PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07B 61/00, C07C 255/41, 323/32, C07D 213/40, B01J 19/00, C07D 317/58, C07C 255/60, C12Q 1/48, C07D 333/20, B01L 3/14, C07D 307/52, G06K 19/06	A2	(11) International Publication Number: WO 97/49653 (43) International Publication Date: 31 December 1997 (31.12.97)

(21) International Application Number: PCT/US97/11035

(22) International Filing Date: 24 June 1997 (24.06.97)

(30) Priority Data:

60/020,706	24 June 1996 (24.06.96)	US
08/711,426	5 September 1996 (05.09.96)	US
08/709,435	6 September 1996 (06.09.96)	US
08/723,423	30 September 1996 (30.09.96)	US

(71) Applicant *(for all designated States except US)*: IRORI [US/US]; Suite 100, 11025 North Torrey Pines Road, La Jolla, CA 92037 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: XIAO, Xiao-Yi [CN/US]; 4951 Riding Ridge Road, San Diego, CA 92130 (US). SHI, Shuhao [US/US]; 4178 Decoro Street #50, San Diego, CA 92122 (US). PARANDOOSH, Zahra [US/US]; 4626 Exbury Court, San Diego, CA 92130 (US). NOVA, Michael, P. [US/US]; 16428 La Gracia, Rancho Santa Fe, CA 92067 (US).

(74) Agent: SEIDMAN, Stephanie, L.; Brown Martin Haller & McClain, 1660 Union Street, San Diego, CA 92101-2926 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: SOLID PHASE TYRPHOSTIN LIBRARY LINKED TO MATRICES WITH MEMORIES

(57) Abstract

Combinations, called matrices with memories, of matrix materials with memories that are remotely addressable or remotely programmable recording devices or are associated with imprinted symbology are provided. In particular, combinations of matrix materials, memories, and linked molecules that are tyrphostin analogs are provided. The matrix materials are those that are used in as supports in solid phase chemical and biochemical syntheses, immunoassays and hybridization reactions. The matrix materials may additionally include fluophors or other luminescent moieties to produce luminescing matrices with memories. The memories include electronic and optical storage media and also include optical memories, such as bar codes and other machine-readable codes. By virtue of this combination, molecules and biological particles, such as the tyrphostin compounds, that are in proximity or in physical contact with the matrix combination can be labeled by programming the memory with identifying information and can be identified by retrieving the stored information. The tyrphostin compounds, as well as methods of preparing the compounds, are also provided.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav	TM	Turkmenistan
BF	Burkina Faso	GR	Greece		Republic of Macedonia	TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

SOLID PHASE TYRPHOSTIN LIBRARY LINKED TO MATRICES WITH MEMORIES

RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional application Serial No. 60/020,706, filed June 24, 1996. To the extent permitted, the subject matter of the provisional application is herein incorporated in its entirety by reference thereto.

This application is related to U.S. application Serial No. 08/826,253 entitled "MATRICES WITH MEMORIES IN AUTOMATED DRUG DISCOVERY AND UNITS THEREFOR, filed March 27, 1997, by Nova, Michael Phillip, Lillig, John, Parandoosh, Zahra, Karunaratne, Kanchanka Sanjaya Gunesekera, O'Neil, Donald, Czarnik, Anthony, Zhao, Chanfeng, Ewing, William, David, Gary, Satoda, Yozo and Potash, Hanan.

This application is also related to U.S. application Serial No. application No. (6444-317D) entitled "MATRICES WITH MEMORIES IN AUTOMATED DRUG DISCOVERY AND UNITS THEREFOR", filed January 22, 1997, by Nova, Michael Phillip, Lillig, John, Parandoosh, Zahra, Karunaratne, Kanchanka Sanjaya Gunesekera, O'Neil, Donald Czarnik, Anthony, Zhao, Chanfeng, Ewing, William, David, Gary Satoda, Yozo and Potash, Hanan.

This application is also related to U.S. application Serial No. application No. 08/741,685 entitled "MATRICES WITH MEMORIES IN AUTOMATED DRUG DISCOVERY AND UNITS THEREFOR", filed October 31, 1996, by Nova, Michael Phillip, Lillig, John, Karunaratne, Kanchanka Sanjaya Gunesekera, Yozo Satoda, and Hanan Potash, and U.S. application Serial No. application No. 08/743,984 entitled "SORTING MATRICES WITH MEMORIES, SENSORS WITH MEMORIES AND USES THEREOF", filed October 28, 1996, by Nova, Michael Phillip, Lillig, John, Parandoosh, Zahra, Karunaratne, Kanchanka Sanjaya Gunesekera, Satoda, Yozo, Sargent, Bradley, Zhao, Chanfeng, Xiao, Xiao-Yi, and Potash, Hanan. This application is also related to U.S. application Serial No. 08/726,703, entitled

"SORTING MATRICES WITH MEMORIES, SENSORS WITH MEMORIES AND USES THEREOF", filed October 7, 1996, by Nova, Michael Phillip, Lillig, John, Karunaratne, Kanchanka, Sanjaya Gunesekera, Satoda, Yozo and Sargent, Bradley; and published International PCT application No. PCT/US96/15999 entitled "MATRICES WITTH MEMORIES AND USES, SENSORS WITH MEMORIES AND USES THEREOF", filed as an International PCT application in the US/RO on October 3, 1996. This application is also related to U.S. application Serial No. 08/723,423 entitled "MATRICES WITH MEMORIES AND USES THEREOF", filed on September 30, 1996, by Michael P. Nova, Zahra Parandoosh, Andrew E. Senyei, Xiao-Yi Xiao, Gary S. David, Yozo Satoda, Chanfeng Zhao and Hanan Potash. This application is also related to U.S. application Serial No. 08/709,435 entitled "MATRICES WITH MEMORIES AND USES THEREOF", filed on September 6, 1996, by Michael P. Nova, Zahra Parandoosh, Andrew E. Senyei, Xiao-Yi Xiao, Gary S. David, Yozo Satoda, Chanfeng Zhao and Hanan Potash. This application is also related to U.S. application Serial No. 08/711,426, entitled "MATRICES WITH MEMORIES AND USES THEREOF", filed on September 5, 1996, by Michael P. Nova, Zahra Parandoosh, Andrew E. Senyei, Xiao-Yi Xiao, Gary S. David, Yozo Satoda, Chanfeng Zhao and Hanan Potash. This application also related to U.S. application Serial No. 08/669,252, entitled "REMOTELY PROGRAMMABLE MATRICES WITH MEMORIES AND USES THEREOF, filed on June 24, 1996, by Michael P. Nova, Andrew E. Senyei, Zahra Parandoosh, Gary S. David, Hanan Potash and Xiao-Yi Xiao.

This application is related to U.S. application Serial No. 08/633,410, entitled "MATRICES WITH MEMORIES AND USES THEREOF", filed on June 10, 1996, by Michael P. Nova, Andrew E. Senyei, Zahra Parandoosh, Gary S. David, Hanan Potash and Xiao-Yi Xiao; published International PCT application No. PCT/US96/06145 which designates the U.S. and which was filed on April 25, 1996; and U.S. application Serial No. 08/639,813, filed April 2, 1996, entitled "REMOTELY PROGRAMMABLE MATRICES WITH

MEMORIES AND USES THEREOF ", by Michael P. Nova, Andrew E. Senyei, Zahra Parandoosh and Gary S. David.

This application is also related to U.S. application Serial No. 08/567,746, filed December 5, 1995, entitled "REMOTELY PROGRAMMABLE MATRICES WITH MEMORIES AND USES THEREOF ", by Michael P. Nova, Andrew E. Senyei, Zahra Parandoosh and Gary S. David; and U.S. application Serial No. 08/538,387, filed October 3, 1995, entitled "REMOTELY PROGRAMMABLE MATRICES WITH MEMORIES", by Michael P. Nova, Andrew E. Senyei, and Gary S. David. This application is also related to each of U.S. application Serial Nos. 08/428,662, 08/480,147, 08/484,486, 08/484,504, 08/480,196, and 08/473,660.

U.S. application Serial No. 08/826,253 is a continuation-in-part of U.S. application Serial No. (atty. dkt. no. 6444-317D), which is a continuation-in-part of U.S. application Serial No. 08/741,685, which is a continuation-in-part of U.S. application Serial No. application No. 08/743,984, which is a continuation-in-part of U.S. application Serial No. application No. 08/726,703, which is a continuation-in-part of International PCT application No. PCT/US96/15999, which is a continuation-in-part of U.S. application Serial No. 08/723,423, which is a continuation-in-part U.S. application Serial No. 08/709,435, which is a continuation-in-part of U.S. application Serial No. 08/711,426, which is a continuation-in-part of U.S. application Serial No. No. 08/669,252, which is a continuation-in-part of U.S. application Serial No. 08/633,410 , which is a continuation-in-part of International PCT application No. PCT/US96/06145, which is a continuation-in-part of U.S. application Serial No. 08/639,813, filed 4/2/96. U.S. application Serial No. attorney dkt. No. 08/726,703 is a continuation-in-part of each of U.S. application Serial No. 08/711,426, filed 9/5/96; U.S. application Serial No. 08//709,435, filed 9/6/96;U.S. application Serial No. 08/723,423 filed 9/30/96; and International PCT application No. PCT/US96/15999, filed 10/3/96. U.S. application Serial Nos. No.

08/711,426, 08/709,435, 08/669,252 and 08/633,410 are each a continuation in-part of International PCT application No. PCT/US96/06145, which in turn is a continuation-in-part of U.S. application Serial No. 08/639,813, which application is a continuation-in-part of U.S. application Serial No. 08/567,746, which application is a continuation-in-part of U.S. application Serial No. 08/538,387, filed October 3, 1995, which in turn is a continuation-in-part of U.S. application Serial Nos. 08/480,147, 08/484,486, 08/484,504, 08/480,196 and 08/473,660, each filed June 7, 1995, and each entitled, "REMOTELY PROGRAMMABLE MATRICES WITH MEMORIES". U.S. application Serial No. 08/669,252 is a continuation-in-part of 08/633,410 and each of the preceding cases.

For U.S. national stage purposes, the subject matter of each of above-noted U.S. applications and International PCT applications is incorporated herein by reference in its entirety. The subject matter of each of U.S. application Serial Nos. 08/379,923, now U.S. Patent No. 5,583,810, and 08/322,644 also is incorporated herein its entirety.

FIELD OF THE INVENTION

The present invention relates to the application of information and data storage and retrieval technology to molecular tracking and identification, and to the preparation of compounds that modulate, particularly inhibit the activity of protein tyrosine kinase using the information and data storage technology. In particular, libraries of tyrphostin compounds and methods for preparing the libraries are provided. Screening assays for identifying active compounds in the libraries are also provided. The libraries are electronically labeled for directed sorting and screening. Also provided are tyrphostin compounds useful as modulators of tyrosine kinase activity as well as pharmaceutical compositions containing the compounds. The invention further relates to methods of treating diseases associated with altered tyrosine kinase activities, such as neoplastic diseases. In another aspect,

the invention relates to methods of evaluating the cancer cell growth inhibitory properties of compounds.

BACKGROUND OF THE INVENTION

Drug Discovery

Drug discovery relies on the ability to identify compounds that interact with a selected target, such as cells, an antibody, receptor, enzyme, transcription factor or the like. Traditional drug discovery relied on collections or "libraries" obtained from proprietary databases of compounds accumulated over many years, natural products, fermentation broths, and rational drug design. Recent advances in molecular biology, chemistry and automation have resulted in the development of rapid, High throughput screening (HTS) protocols to screen these collection. In connection with HTS, methods for generating molecular diversity and for detecting, identifying and quantifying biological or chemical material have been developed. These advances have been facilitated by fundamental developments in chemistry, including the development of highly sensitive analytical methods, solid state chemical synthesis, and sensitive and specific biological assay systems.

Analyses of biological interactions and chemical reactions, however, require the use of labels or tags to track and identify the results of such analyses. Typically biological reactions, such as binding, catalytic, hybridization and signaling reactions, are monitored by labels, such as radioactive, fluorescent, photoabsorptive, luminescent and other such labels, or by direct or indirect enzyme labels. Chemical reactions are also monitored by direct or indirect means, such as by linking the reactions to a second reaction in which a colored, fluorescent, chemiluminescent or other such product results. These analytical methods, however, are often time consuming, tedious and, when practiced in vivo, invasive. In addition, each reaction is typically measured individually, in a separate assay. There is, thus, a need to develop alternative and convenient methods for tracking and

identifying analytes in biological interactions and the reactants and products of chemical reactions.

Combinatorial libraries

The provision and maintenance of compounds to support HTS have become critical. New methods for the lead generation and lead optimization have emerged to address this need for diversity. Among these methods is combinatorial chemistry, which has become a powerful tool in drug discovery and materials science. Methods and strategies for generating diverse libraries, primarily peptide- and nucleotide-based oligomer libraries, have been developed using molecular biology methods and/or simultaneous chemical synthesis methodologies. The resulting combinatorial libraries potentially contain millions of pharmaceutically relevant compounds and that can be screened to identify compounds that exhibit a selected activity.

The libraries fall into roughly three categories: fusion-protein-displayed peptide libraries in which random peptides or proteins are presented on the surface of phage particles or proteins expressed from plasmids; support-bound synthetic chemical libraries in which individual compounds or mixtures of compounds are presented on insoluble matrices, such as resin beads [see, e.g., Lam et al. (1991) Nature 354:82-84] and cotton supports [see, e.g., Eichler et al. (1993) Biochemistry 32:11035-11041]; and methods in which the compounds are used in solution [see, e.g., Houghten et al. (1991) Nature 354:84-86, Houghten et al. (1992) BioTechniques 313:412-421; and Scott et al. (1994) Curr. Opin. Biotechnol. 5:40-48]. There are numerous examples of synthetic peptide and oligonucleotide combinatorial libraries. The present direction in this area is to produce combinatorial libraries that contain non-peptidic small organic molecules. Such libraries are based on either a basis set of monomers that can be combined to form mixtures of diverse organic molecules or that can be combined to form a library based upon a selected pharmacophore monomer.

There are three critical aspects in any combinatorial library: (i) the chemical units of which the library is composed; (ii) generation and categorization of the library, and (iii) identification of library members that interact with the target of interest, and tracking intermediary synthesis products and the multitude of molecules in a single vessel. The generation of such libraries often relies on the use of solid phase synthesis methods, as well as solution phase methods, to produce collections containing tens of millions of compounds that can be screened in diagnostically or pharmacologically relevant in vitro assay systems. In generating large numbers of diverse molecules by stepwise synthesis, the resulting library is a complex mixture in which a particular compound is present at very low concentrations, so that it is difficult or impossible to determine its chemical structure. Various methods exist for ordered synthesis by sequential addition of particular moieties, or by identifying molecules based on spacial positioning on a chip. These methods are cumbersome and ultimately impossible to apply to highly diverse and large libraries. Identification of library members that interact with a target of interest, and tracking intermediary synthesis products and the multitude of molecules in a single vessel is also a problem. While considerable efforts have been devoted to the development of solid support chemistry, the choice of methods for structural elucidation has been limited to spatial addressing, mixture deconvolution, direct microanalysis and chemical tagging [see, e.g., Metzger et al. (1994) Jung, Anal. Biochem. 219:261; Brown et al. (1995) Mol. Diversity 1:4; Youngquist et al. (1995) J. Am. Chem. Soc. 177:3900; Brummel et al. (1994) Science 264:399; Brenner et al. (1992) Proc. Natl. Acad. Sci. U.S.A. 89: 5381; Needles et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:10700; Ohimeyer et al.Proc. Natl. Acad. Sci. U.S.A. 90: 10922; Eckes (1994) Angew. Chem. Int. Ed. Engl. 33:1573; Ni et al. (1996) J. Med. Chem. 39:1601]. Tagging, especially non-chemical, non-invasive tagging, is potentially the most efficient and reliable structural tracking method.

Bar codes and identification

Automated identification of articles using bar codes in the availability of the integrated circuit technology and computing power at reasonable costs. Such codes are typically used to track and identify consumer goods and other articles of manufacture. One of the first scanners capable of reading a bar code was installed at a supermarket in 1974, and by 1980 more than 90% of all grocery items carried a bar code by 1980. By December 1985, more than 12,000 grocery stores were equipped with scanner checkout systems [See, e.g., Harmon et al. (1989) *Reading Between the Lines-An Introduction to Bar Code Technology*, Helmers Publishing, Inc. 1989]. Bar codes have also been used in other applications, including other inventory control systems and for identification and characterization of responses to mass advertising efforts.

By electro-optically scanning the symbol on an item and generating a corresponding signal, it is possible in an associated computer whose memory has digitally stored therein the full range of items, to compare the signal derived from the scanned symbol with the stored information. When a match is found, the identity of the item and associated information, such as, in the instance of consumer goods, its price. Thus computer technology is exploited to facilitate identification procedures using machine-readable identifiers.

Bar codes are typically read using lasers that scan from left to right, right to left, or in both directions (or other directions) across a field of alternating dark bars and reflective spaces of varying widths. Multiple scans are typically employed to minimize data errors. Because of the multiplicity of bars and spaces required for each alphanumeric character, bar codes generally require a relatively large space to convey a small amount of data. For instance, each character in the bar code system known as Code 39 requires five bars and four spaces. A high density Code 39 field corresponds to only 9.4 characters per inch. Universal Product Codes

(UPCs) are another common bar code used primarily in the retail grocery trade and contain a relatively large number of bars and spaces which allow for error checking, parity checking and reduction of errors caused by manual scanning of articles in grocery stores. They accordingly require even larger space for conveyance of character information. The Codabar code, which has been developed by Pitney Bowes and is used in retail price labeling systems and by Federal Express, is a self-checking code. Each character is represented by a stand-alone group of four bars and three interleaving spaces. Federal Express uses an eleven digit Codabar symbol on each airbill to process more than 450,000 packages per night. Other codes use varying bar and space techniques to represent characters. Because of error checking requirements and for other reasons, however, the space required to place a bar code on an article is relatively large.

In addition to the large surface area required for the series of bars and spaces that form a typical bar code symbol, the code must be placed on a background that has a high reflectance level. The high level of contrast, or reflectivity ratio, between the dark bars and the reflective spaces, allows the optical sensor in the reader to discern clearly and dependably the transitions between the bars and spaces in the symbol. Ideally, the printed bar should be observed as perfectly black and the spaces should be perfectly reflective. Because those ideal conditions are seldom possible, the industry typically requires that labeling media reflect at least 70% of incident light energy. Surface reflectivity and thus quality of the media on which the bar code is placed directly affects the successful use of the bar code on that media. Additionally, the media cannot be overly transparent or translucent, since those characteristics can attenuate reflected light. Accordingly, only limited types of highly reflective media may be used for placement of bar codes. Space requirements for bar codes further include a "quiet zone" that surrounds the field of bars and spaces. In many codes, this quiet zone

constitutes a border around the code symbol, thus requiring even more space for the bar code.

Bar coding also requires very precise print methods. Assuming that the printing operation is capable of printing the required density to achieve the 70% reflectance ratio, careful attention must be paid to additional major factors that influence the bar code effectiveness. Those include ink spread/shrinkage; ink voids/specks; ink smearing; non-uniformity of ink; bar/space width tolerances; edge roughness and similar factors that must be closely controlled to ensure that the symbol will be easily scannable. In other words, the printer must pay careful attention to using paper or other media that displays the correct absorption properties properly inking the ribbon; carefully controlling hammer pressure; keeping the printhead and paper clean; properly wetting the paper and curing the ink; and maintaining proper adjustment of the printhead control mechanism. These printing details create additional problems and expenses, particularly for placement of bar code symbols on smaller items such as coupons and mail pieces.

"Bar codes" containing an array of marks of any desired size and shape that are arranged in a reference context or frame of one or more columns and one or more rows, together with a reference marker and a reference cue have also been developed [see, U.S. Patent No. 5,128,528]. The number of rows corresponds to the number of characters contained in the symbology selected for the array. For example, an array that is capable of conveying all the letters of the English language and ten numeral symbols could use 36 rows. The number of columns in the matrix could corresponds to the number of characters desired to be conveyed. The roles of the rows and columns in the reference frame may be reversed if desired. In the preferred embodiment, each column contains one or more dots corresponding to the character which is desired to be conveyed in that column. The reference marker and reference cue may be formed of one

shape, of two marks, or according to any other desired arrangement that allows interpretation of the matrix at any desired attitude with respect to the imaging equipment. The reference cue may form a part of the reference marker, or an information dot, if desired.

Thus, there are numerous types of bar codes, codes and methodologies for use available. Bar coding and other coding technology, however, remains to be fully exploited in areas outside the consumer products domain. Furthermore, other types of optical memories have not been exploited in any industry.

High Throughput Screening

In addition, exploitation of this diversity requires development of methods for rapidly screening compounds. Advances in instrumentation, molecular biology and protein chemistry and the adaptation of biochemical activity screens into microplate formats, has made it possible to screen of large numbers of compounds. Also, because compound screening has been successful in areas of significance for the pharmaceutical industry, high throughput screening (HTS) protocols have assumed importance. Presently, there are hundreds of HTS systems operating throughout the world, which are used, not only for compound screening for drug discovery, but also for immunoassays, cell-based assays and receptor-binding assays.

An essential element of high throughput screening for drug discovery process and areas in which molecules are identified and tracked, is the ability to extract the information made available during synthesis and screening of a library, identification of the active components of intermediary structures, and the reactants and products of assays. While there are several techniques for identification of intermediary products and final products, nanosequencing protocols that provide exact structures are only applicable on mass to naturally occurring linear oligomers such as peptides and amino acids. Mass spectrographic [MS] analysis is sufficiently sensitive to determine the exact mass and fragmentation patterns of

individual synthesis steps, but complex analytical mass spectrographic strategies are not readily automated nor conveniently performed. Also, mass spectrographic analysis provides at best simple connectivity information, but no stereoisomeric information, and generally cannot discriminate among isomeric monomers. Another problem with mass spectrographic analysis is that it requires pure compounds; structural determinations on complex mixtures is either difficult or impossible. Finally, mass spectrographic analysis is tedious and time consuming. Thus, although there are a multitude of solutions to the generation of libraries and to screening protocols, there are no ideal solutions to the problems of identification, tracking and categorization.

These problems arise in any screening or analytical process in which large numbers of molecules or biological entities are screened. In any system, once a desired molecule(s) has been isolated, it must be identified. Simple means for identification do not exist. Because of the problems inherent in any labeling procedure, it would be desirable to have alternative means for tracking and quantitating chemical and biological reactions during synthesis and/or screening processes, and for automating such tracking and quantitating.

Preparation and Identification of tyrosine kinase inhibitors

One area of drug discovery that has been the focus of intense research is the search for modulators of tyrosine kinase activity. Tyrosine kinases, which play a primary role in signal transduction in cells, regulate cell proliferation, cell differentiation and signaling processes in cells of the immune system. Protein tyrosine kinases occur as membrane-bound receptors and as cytoplasmic proteins. Many oncogene products exhibit protein tyrosine kinase (PTK) activity. They are involved in regulating a variety of cellular functions and responses, including, cytokine response, antigen-dependent immune responses, cellular transformation by RNA viruses, oncogenesis and mitogenesis. These functions and responses are

regulated via intracellular signaling pathways, including Ras, phospatidylinositol 3 kinase, phospholipase and mitogen activated pathway. Activation results in auto-phosphorylation of a tyrosine residue in the protein tyrosine kinase, thereby facilitating interaction of protein substrates with the protein tyrosine kinase active site, which results in phosphorylation of tyrosine residues in the protein substrates. The protein substrates include cytosolic signalling molecules that are activated or inactivated when phosphorylated. Activation of these substrates results in a cascade of intracellular reactions leading to activation or regulation of cellular functions via activation or inhibition of gene expression.

Tyrosine kinase activity has been implicated in many and proliferative diseases and cancers and other diseases, including psoriasis, atherosclerosis, restenosis, and pulmonary fibrosis. The proliferation and directed migration of vascular smooth muscle cells are important components in processes, such as restenosis and atherosclerosis. Thus, modulation of tyrosine kinase activity, has been targeted for drug development. For example, tyrosine kinase inhibitors have been shown to reduce or prevent cartilage degradation, such as that associated with osteoarthritis, and hyperproliferative disorders of the epithelium. Tyrosine kinase inhibitors have also been shown to inhibit growth factor, such as epidermal growth factor (EGF), fibroblast growth factors (FGFs) and platelet derived growth factors (PDGFs), dependent cell proliferation, and, thus, have use in treating tumors, restenosis and atherosclerosis.

Thus, PTK inhibitors are targets for selective therapies, and early drug screening efforts focused on screening for such compounds. Among those identified was erbstatin (Umezawa et al. (1986) Antibiotics 39:170), which inhibits the autophosphorylation of the EGF receptor in A431 epidermoid carcinoma cells.

Tyrphostins

Tyrphostins, are derivative synthetics of erbstatin, which are a prototype tyrosine analogue, have been reported to block phosphorylation of tyrosine. Tyrphostins is the name given to the class of benzylidene malononitriles that have demonstrated inhibition of various protein tyrosine kinase (PTK) activities. The tyrphostin structural motif is an alkene with cis-oriental nitrile and oxygenated benzene groups. While hydroxylation is the most reported mode of oxygenation, alkoxylated derivatives are known that reveal similar PTK activity.

The tyrphostin class of tyrosine kinase inhibitors have been found to block the proliferation of squamous cell carcinoma cells in in vitro and in vivo (see, e.g., Yoneda, et al. (1991) "The Antiproliferative Effects of Tyrosine Kinase Inhibitors Tyrphostins on a Human Squamous Cell Carcinoma In Vitro and in Nude Mice," Cancer Res. 51:4430-4435 (1991); Lyall et al. (1989) " Tyrphostins Inhibit Epidermal Growth Factor (EGF) Receptor Tyrosine Kinase Activity in Living Cells and EGF-stimulated Cell Proliferation,"J. Biol. Chem. 264:14503-14509. Tyrphostins also inhibit the epidermal growth factor (EGF)-dependent growth of A431 cells in vitro (Gazit et al. J. Med. Chem. 1989;32:2344; Gazit et al. J. Med. Chem. 1991;34:1896) and inhibit EGF dependent cell proliferation at concentrations showing little toxicity (Bilder et al. (1991) Tyrphostins Inhibit PDGF-Induced DNA Synthesis and Associated Early Events in Smooth Muscle Cells, American Journal of Physiology 260:C721-C730. The tyrphostin derivative designated AG490 has been reported to block leukemic cell growth by inducing apoptosis without deleterious effects on normal haematopoiesis (see, e.g., Meydan et al. (1996) Nature 379:645-648). AG490 has also been reported to inhibit the growth of chronic myelogenous leukemia (CML) K562 cells.

Thus, compounds that inhibit tyrosine kinase activity have pharmaceutical use, as for example, anti-neoplastic and anti-proliferative

agents, and also as tools for investigation of signal transduction protein tyrosine kinases. In particular, such compounds will have use as candidates for treating cancers, including leukemias and Kaposi's sarcoma, psoriasis, postsurgical vascular stenosis and restenosis. Therefore, there is a need for compounds that are effectively inhibit PTKs for use as candidates for treatment of disorders in which PTKs play a role. Therapeutic approaches based on cell signaling, however, present the challenge of finding compounds that selectively react with a target receptor. Since PTK's act on structurally similar substrates (at least at the tyrosine level), a means to identify inhibitors with useful selectivities must be developed. Also, because the structures PTK's are not available of use in inhibition design, and would not assure assistance even if they were, there is a need to develop methods of synthesizing large numbers of structurally distinct compounds as potential candidates as tyrosine kinase modulators. Furthermore, there is a need for means of separating, tracking, and identifying each distinct compound prepared by such methods. Additionally, there is a need for automated methods for rapidly evaluating the cell growth inhibitory activities of large numbers of compounds.

Therefore, it is an object herein to provide methods for synthesizing diverse mixtures of compounds that have PTK inhibitory activity, identifying, tracking and categorizing the components of the mixtures of the diverse molecules and identifying clinical candidates. It is also an object herein to provide means for such identification, tracking and categorization and to provide assays, diagnostics and screening protocols that use such means. It is of particular interest herein to provide PTK inhibitory compounds and the means to track and identify the compounds and to perform screening, particularly, HTS protocols.

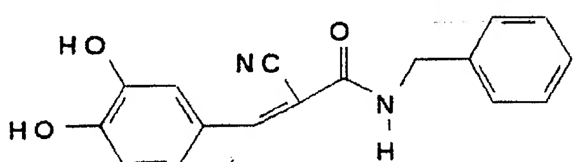
In particular, it is an object herein to provide methods for synthesis, identification, tracking and categorization of related, yet diverse, molecules that inhibit PTK activity. It is also an object herein to provide compounds useful as anti-neoplastic agents. Another object herein is to provide rapid, automatable methods for evaluating the cell growth inhibitory activity of compounds.

SUMMARY OF THE INVENTION

Combinations of matrix materials with programmable data storage or recording devices or other recording means, herein referred to as memories, and assays using these combinations are provided. These combinations are referred to herein as matrices with memories. The matrix materials serve as the support matrix for synthesis of compounds. A plurality of the matrices with memories comprising a library are provided.

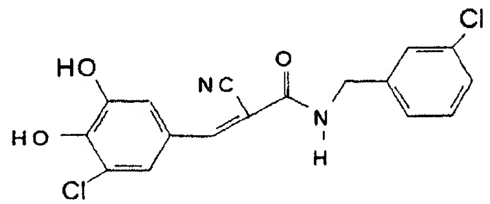
Provided herein are libraries of tyrphostin analogs, particularly analogs of AG490, a method of synthesis of the libraries and a method for screening the libraries.

Tyrphostin analog compounds are also provided. The compounds provided herein are analogs of tyrphostin AG490, which has the structure:



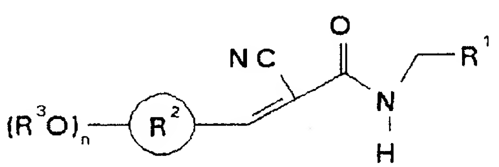
AG 490

and analogs thereof produced by the synthetic method provided herein are prepared. Analogs of typrohstin AG490 provided herein have formula (I). It is understood that, when considering formula (I) as a description of compounds, AG490 as well as the compound:



(see, Kaur et al. (1994) Anti-cancer drugs 5:213-233), are excluded from general formula (I) as set forth below.

The analogs provided herein have formula (I)



in which:

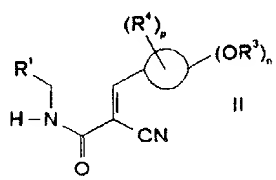
R^1 is selected from the alkyl, which may be straight or branched or cyclized containing one ring or two fused rings, or mixtures thereof, containing preferably 1 to 15 carbons, more preferably 1 to 6, and is more preferably aryl or heteroaryl, containing preferably from 5 to 7 members in the ring, and is more preferably phenyl, thienyl, furyl, pyrrolyl, phenyl, pyridinyl, morpholino, or pyrimidinyl; R^1 is unsubstituted or is substituted with one or more substituents selected from alkyl, cyano, halide, alkoxy, alkoxyalkyl, alkyl, thioxy, alkylthioxy, haloalky, OH, and amino;

R^2 is selected from the alkyl, which may be straight or branched or cyclized containing one ring or two fused rings, or mixtures thereof, containing preferably 1 to 15 carbons, more preferably 1 to 6, and is more

preferably aryl or heteroaryl, containing preferably from 5 to 7 members in the ring, and is more preferably phenyl, thienyl, furyl, pyrrolyl, phenyl, pyridinyl, morpholino, or pyrimidinyl; R^2 is unsubstituted or is substituted with one or more substituents selected from $(R^4)_p$, in which each R^4 is independently selected from alkyl, cyano, halide, alkoxy, alkoxyalkyl, alkyl, thioxy, alkylthioxy, haloalky, OH, and amino, where R^4 is preferably OH or halide or lower alkyl or alkoxy, and n is preferably 0 to 2; and

R^3 is H, $(CH_3)(CH_2)_qC(O)$ where q is 0 to 3, includes aryl or heteroaryl ring preferably containing 5 to 7 members in the ring, which is unsubstituted or substituted, preferably with the substituents set forth for R^4, and is of formula aryl-$(CH_2)_qC(O)$ or heteroaryl-$(CH_2)_qC(O)$, and preferably is acetyl, or benzoyl, or is H.

Preferred among those compounds are those in which the resulting compounds have formula (II):



II

where ◯ is an aryl or heteroaryl group

in which:

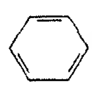
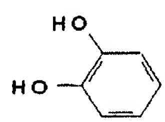
n is 0 to 3, preferably 1 or 2 and p is 0 to 3, preferably 1 or 2, as long as n + p is not greater than 5;

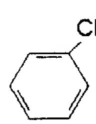
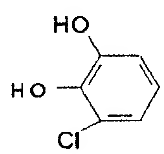
R^2 is aryl or heteroaryl contain one or two fused rings, preferably one ring with 5 to 7 members, preferably 5 or 6 members, more preferably phenyl or pyrimidinyl;

each R^4 is independently selected from among H, alkyl, cyano, halide, alkoxy, alkoxyalkyl, alkyl, thioxy, alkylthioxy, haloalky, OH, nitro and amino; and

R^3 is selected from H, alkvl, acetyl, $CH_3(CH_2)_qC(O)$ where q is 0 to 3, aryl-$(CH_2)_qC(O)$ or heteroaryl-$(CH_2)_qC(O)$, which are unsubstituted or

substituted with one or more substituents, such as R^4, which is preferably halide, lower alkyl, lower alkoxy, nitro, and preferably contain 5 to 7 members in the ring and is of formula aryl-C(O) or heteraryl-C(O). R^3 is more preferably acetyl, or includes an aryl or heteroaryl containing 5 or 6 members in the ring and one heteroatom, selected from among S, O, N, or aryl, containing 5 to 7 members in the ring or aryl, in which the aryl group is preferably a 5 or 6-membered ring, preferably phenyl and the heteroaryl is preferably selected from thienyl, furyl, pyrrolyl, phenyl, pyridinyl, morpholino, or pyrimidinyl. Even more preferably R^3 is acetyl or benzoyl, which is unsubstituted or substituted with R^4. In all instances, if R^3 is H [i.e. BB3, which is R^3X (see, e.g., FIGURES 35-39)] is nothing, then R^1 and R^2 cannot be:

 and  , respectively, or

 and  , respectively.

Compounds of formula (II) where R^2 is a 5 or 6-membered heteroaryl group are also among those preferred herein. Presently more preferred are compounds in which R^2 is phenyl.

Preferred selections for each of R^1, R^2 and R^3 will be apparent from the specific compounds and libraries provided herein. Any selection of each of these in the specific compounds described herein will be included in the above definitions. Additional diversity among the resulting compounds is achieved by altering the position of R^3. When R^3 in formula (I) is hydrogen, then the estrification step in which BB3 is added is not included.

Preferred selections for R^1, R^2 and R^3 can be determined from synthetic scheme I set forth in FIGURE 35, in which each substitutent can be determined from the starting materials BB1, BB2 and BB3. BB1 is R^1CHO,

where BB1 is any aldehyde, preferably an aromatic or heterocyclic aldehye; BB2 is any aldehyde, preferably an aromatic or heterocyclic aldehyde, more preferably an aromatic or heterocyclic aldehyde with one or more hydroxy groups, particularly of formula $OHC\text{-}R^2\text{-}(OH)_n$ were n is 0-3, inclusive, and more preferably BB2 is a substituted phenol aldehyde; and BB3 is an alkylating or acylating reagent, particularly an alkylating or acylating reagent with aromatic or heterocyclic ring(s), preferably of formula R^3-X, in which X is a leaving group, particularly halide, preferably Cl, R^3 is as defined above, and is preferably, Ph-C(O), $CH_3(CH_2)_nC(O)$, where n is 0 to 3, or R^4-substituted Ph-C(O)). When R^3 in formula (I) is H, then the esterification step in which BB3 is added is not included, and BB3 is nothing.

Preferred selections for each of BB1, BB2 and BB3 in the exemplified libraries are set forth in FIGURES 35-39. Combinations and subsets of the substituents for each building block (BB) may be used in the methods herein to generate compounds and libraries containing such compounds.

The resulting compounds and pharmaceutical compositions containing a compound of Formula (I) are also provided. Libraries comprising a plurality of the compounds are provided. The libraries preferably include the compounds linked to the matrix or associated with the memory so that the identity of the compounds can be readily ascertained and tracked throughout the drug discovery process.

Thus, libraries containing combinations of matrices with memories and compounds of Formula (I) linked thereto or associated therewith or proximate thereto are provided. The combinations provided herein have a multiplicity of applications, including for combinatorial synthesis, isolation and purification of the target compounds, capture and detection of macromolecules for analytical purposes, high throughput screening.

The compounds may be prepared on solid supports that do not contain memories as well as and preferably on the matrices with memories. An exemplary synthetic scheme for preparation of the compounds on a

matrix with memory is set forth in FIGURE 35, with a specific embodiment detailed in FIGURE 36. Building blocks for exemplary libraries are set forth in FIGURES 35-39.

The resulting combinations of memories with matrices and the tyrphostin analog compoudns are particularly advantageous for use in multianalyte analyses, assays in which a electromagnetic signal is generated by the reactants or products in the assay, for use in homogeneous assays, and for use in multiplexed protocols.

Combinations of matrices with memories with compounds of formula (I) linked thereto are provided. The memories may be electromagnetic memories or optical memories, including optical bar codes, as described herein. Matrices that have an engraved code, herein referred to as matrices with codes or optical memory devices [OMDs], in combination with the compounds of Formula (I) are also provided. The materials are encoded with identifying information and/or any other information of interest. By virtue of this memory with matrix combination the compounds provided herein can be identified, tracked, sorted and screened and isolated.

Compounds of Formula (I) provided herein possess anti-tumor properties. As such, the compounds can have inhibitory effects on the survival of cancer cells. The ability of compounds to inhibit cancer cell survival can be demonstrated in a variety of ways. Compounds provided herein yield positive results in one or more assays designed to evaluate the cancer cell growth inhibitory properties of compounds. For example, using methods provided herein, compounds can be evaluated for the ability to inhibit cell growth and/or proliferation or induce differentiation.

Assays for screening and identifying active compounds from the libraries of compounds of formula (I) are provided. Also provided are scintillation proximity assays, HTRF, FP, FET and FRET assays in which the memories are in proximity with or are in physical contact with the matrix that contains scintillant for detecting proximate radionucleotide signals or

fluorescence. In addition, embodiments that include a memory device that also detects occurrence of a reaction are provided.

Assay methods, which are preferably automated, provided herein for evaluating the cell growth inhibitory activity of compounds permit identification of suitable candidate compounds from among the collections of compounds synthesized by the methods herein. The automated versions of the assays permit rapid, high-throughput analysis of large numbers compounds. As such, these methods are not only useful in screening compounds to identify those with desired properties, but also in comparing the relative activities of a large series of compounds and in collecting data required in the detailed characterization of compounds, e.g., for constructing dose-response curves used in calculating IC_{50} values and other such parameters.

A particularly preferred use of the assay methods provided herein is in the evaluation of the cell growth inhibitory activities of compounds associated with matrix combinations and selection of pharmaceutically useful compounds from the library. Such methods combine rapid analytical processes with the ability to readily identify and track compounds displaying desired properties to yield a powerful tool facilitating drug discovery and evaluation.

Screening assays for identifying active members of the libraries are also provided. In one particular embodiment, the screening assays identify compounds that inhibit protein tyrosine kinases (PTKs), particularly, PTKs associated with leukemic cells. The compounds that inhibit PTKs at concentrations as low as tyrphostin AG490 or as effectively as AG490 are selected. The compounds may also be further screened by identifying those that are selectively cytotoxic for targeted cells. Selected compounds will have antiproliferative effects on leukemic cells that are as good as or better than tyrphostin AG490 but will exhibit the same or less cytoxocity on non-targeted cells. Protocols in which all steps, including synthesis and

screening or assaying, are performed on a single platform, a matrix with memory, are provided herein. Also, by selecting appropriate enzymes and substrates the assays can be modified such that compounds with selectivity for different target receptors from AG490, cells, tissues or disorders can be selected. The assay provided herein can be readily adapted to for selection of compounds that exhibit differing selectivities and specificities.

The compounds are preferably linked to support matrices that include memories. Programming of the memories is preferrably effected through electromagnetic tagging. The combinations also can be tagged by imprinting, such as with the two-dimensional bar code described herein, the matrix with identifying information. Programming and reading the memory is effected remotely, preferably using electromagnetic radiation, particularly radio frequency [RF] or radar, microwave, or microwave or energies between RF and microwave, or by reading the imprinted information. Optical memories, either bar coded information or optically encoded memories, such as memories that rely on changes in chemical or physical properties of particular molecules are contemplated herein. Memories may also be remote from the matrix, such as instances in which the memory device is precoded with a mark or identifier or the matrix is encoded with a bar code. The identity [i.e., the mark or code] of each device is written to a memory, which may be a computer or a piece of paper or any recording device, preferably a computer or other such memory device, and information associated with each matrix is stored in the remote memory and linked to the code or other identifier.

The materials are encoded with identifying information and/or any other information of interest. Synthetic protocols and assays using encoded matrix materials are provided. By virtue of this code on the matrix the compounds provided herein or any other molecules, such as antigens, antibodies, ligands, proteins and nucleic acids, and biological particles, such as phage and viral particles and cells, that are associated with, such as in

proximity to or in physical contact with the matrix, can be tagged by programming a memory, such as a memory in a computer, with data correspoding to the encoded identifying information. Programming and reading the memory is effected remotely, preferably using electromagnetic radiation, particularly radio frequency (RF), microwave and between RF and microwave. As a result the identify of compounds linked to the supports can be ascertained by querying the memory and the compounds linked to the supports can be sorted during synthesis using this information, and subsequently screened without requiring chemical or physical analysis of the comopounds.

Compounds of formula (I) modulate the activity of tyrosine kinases, and thereby, if selected against cancer cells, can have inhibitory effects on the survival of cancer cells, particularly leukemic cells such as those that occur in chronic myelogenous leukemia and acute lymphoblastic leukemia. In preferred embodiments, compounds are selected based on their ability to inhibit PTKs in an in vitro assay, particularly the assay provided herein. Among the compounds that have inhibitory, those that have relatively low cytotoxicity on non-cancerous cells, such as NIH 3T3 cells, compared to the selected target tumor cells, are selected as drug candidates. The libraries provided herein may be screened for PTK inhbitory effects against other target cells. The assay provided herein is particularly adaptable therefor by selecting appropriate enzymes and substrates, tyrphostin analogs with other selectivities, such as against FGF receptors or PDGF receptors can be selected.

This, compounds of formula (I) may also have pharmacological activity for use in treating other disorders, including restenosis, proliferative skin and eye disorders, such as psoriasis and diabetic retinopathies, and other tumors, particularly tumors, such as human sqamous cell carcinomas that express high levels of EGF receptors. Any disorder for which a

tyrphostin is a candidate treatment may be treated using compounds from the libraries provided herein.

Methods of treating these disorders, particularly neoplastic diseases, including but not limited to leukemia, are provided herein. The methods use compounds of Formula (I) as chemotherapeutic agents. In particular, methods of treating patients suffering from leukemias and related disorders are effected by administering an effective amount of the pharmaceutical compositions provided herein.

Further provided are solid phase synthetic methods of preparing the compounds of Formula (I) and the libraries. In preferred embodiments, the methods use a polystyrene-based resin suitably derivatized and linked to a linker, preferably an acid cleavable linker, as the solid phase. The resin is preferably coated on a suitable support material, such as a polypropylene or teflon.

Particularly preferred methods of preparing compounds of Formula (I) incorporate steps whereby matrix materials with memories are combined with intermediates in the synthesis process for purposes of identifying the unique R group substitutents in each distinct compound. Thus, each distinct compound synthesized using such methods as provided herein has a unique electromagnetically readable code programmed into the linked memory during synthesis. By virtue of this memory with matrix combination, the compounds of Formula (I) that are associated with, such as in proximity to or in physical contact with the matrix combination, can be identifiably tagged by programming the memory with data corresponding to identifying information and, thus, can be sorted by reading the memories.

Programming and reading the memory is effected remotely, preferably using electromagnetic radiation, particularly radio frequency, microwave or radar. The distinct compounds of Formula (I) that are associated with the matrix combination can be identified by retrieving the stored data points from the memories. Querying the memory will identify the associated

molecules. In particular, the compounds are synthesized on a support and linked preferably via an acid cleavable linker by the steps including addition of an aldehyde (BB1) via a reductive alkylation, followed by a cyanoacetylation, addition of the second aldehyde (BB2) via an aldol condensation, and then an optional esterification (BB3). Following synthesis, the compounds may be stored, assayed while linked to the support, cleaved and then assayed or other subjected to any other suitable protocol.

The compounds are preferably synthesized according the scheme set forth in Figure 35, where BB1 is any aldehyde of formula R^1-CHO, and is preferably an aromatic or heterocyclic aldehyde; BB2 is of formula OCH-R^2-$(OH)_n$ in which n is 0 to 3, and is preferably an aromatic or heterocyclic aldehyde, more preferably an aromatic or heterocyclic aldehyde withone or more substituent hydroxy groups; and BB3, which is optional, has the formula OR^3X, where X is a suitable leaving group, and is any alkylating or acylating reagent, preferably an alkylating or acylating reagent with aromatic or heterocyclic rings. BB3 is preferably acetyl-X, Ph-C(O)X, $CH_3(CH_2)_nC(O)X$, where n is 0 to 3, or sustituted Ph-C(O)X, where X is a suitable leaving group, particularly halide.

The matrix materials [matrices] are any materials that are routinely used in chemical and biochemical synthesis. The matrix materials are typically polymeric materials that are compatible with chemical and biological syntheses and assays. All or a portion of the matrix material is treated to render it suitable for linking compounds. Such matrix materials include, glasses, silicates, celluloses, polystyrenes, polysaccharides, polypropylenes, TEFLON, sand, and synthetic resins and polymers, including acrylamides, particularly cross-linked polymers, cotton, and other such materials. The matrices may be in the form of particles or may be continuous in design, such as a test tube or microplate, 96 well or 384 well or higher density formats or other such microplates and microtiter plates.

Alternatively, the matrices may encase the recording device and have an outer surface that has been rendered suitable, such as by coating with a polymer and/or derivatization, for linking the compounds provided herein, either directly, or preferably via a linker, such as an acid cleavable linker.

The matrices may contain one or a plurality of recording devices. For example, each well or selected wells in the microplate include a memory device in contact therewith or embedded therein. The plates may further contain embedded scintillant or a coating of scintillant [such as FlashPlate™, available from DuPont NEN®, and plates available from Packard, Meriden, CT]. Automated robotic protocols will incorporate such plates for automated multiplexing [performing a series of coupled synthetic and processing steps, typically, though not necessarily on the same platform, i.e. coupling of the chemistry to the biology] including one or more of the following, synthesis, preferably accompanied by writing to the linked memories to identify linked compounds, screening, including using protocols with matrices with memories, and compound identification by querying the memories of matrices associated with the selected compounds.

The matrix material for linking molecules can be particulate of a size that is roughly about 1 to 20 mm^3 [or 1-20 mm in its largest dimension], preferably about 10 mm^3 or smaller, preferably 1 mm^3 or smaller, such as resins typically used for organic syntheses, or a continuous medium, such as a microtiter plate, or other multi-well plate, or plastic or other solid polymeric vial or glass vial or catheter-tube [for drug delivery] or such container or device conventionally used in chemistry and biological syntheses and reactions. In instances in which the matrix is continuous, the data storage device [memory] may be placed in, on, or under the matrix medium or may be embedded in the material of the matrix.

In other embodiments the matrices form a continuous surface, such as a tubular surface encasing the memory device. All or a portion, sufficient for the purposes herein, of the outer surface of the support matrix is

adapted for linking the compounds. In particular, the support surface is treated, such as by radiation grafting of monomers, whereby the surface can be derivatized for linking compounds. Preferred embodiments are the MICROTUBE® microreactors or similar devices described herein. Other preferred embodiments are the MICROKAN® microreactors, described herein, which contain particular matrix materials that are suitably derivatized for linking the compounds.

These devices and microplates and other containers may include a bar code, particularly the two-dimensional optical bar code provided herein in place of or in addition to the electromagnetic tag. For instance, a bar code may be imprinted on the outer surface of the MICROTUBE® microreactor or on the base of each well of a microplate or elsewhere.

As described elsewhere herein, microvessels that are hollow, preferably tubular, devices fabricated from a polymeric material, such as PFTE, EFTE, or other such material as set forth herein, and treated to render the outer surface suitable for linking biological particles or molecules are provided herein. These microvessels include the MICROTUBE® microreactors.

The plates or microreactors or other support matrices may include a bar code, particularly the two-dimensional optical bar code provided herein on the base of each well or elsewhere in place of the memory device or in addition to the memory device. The two-dimensional bar code or other such code is particularly suited for application to each well in a microplate, such as a microtiter plate, that contain 96, 384, 1536 or higher density formats. The bar code may also be used in combination with modules that are fitted into the frames of 96 wells, or higher density formats [such as those available from NUNC, such as NUNC-Immuno Modules, and also sources, such as COSTAR plate strips, and Octavac Filter Strips]. Separate containers or strips of containers are designed to fit into microplate frames. Each such container may be encoded with a bar code so that, upon removal

from the strip, the container, and thereby, its contents or history, may be identified.

In embodiments herein in which the matrices with memories are used in assays, such as scintillation proximity assays [SPA], FP [fluorescence polarization] assays, FET [fluorescent energy transfer] assays, FRET [fluorescent resonance energy transfer] assays and HTRF [homogeneous time-resolved fluorescence] assays, the matrices may be coated with, embedded with or otherwise combined with or in contact with assay material, such as scintillant, fluophore or other fluorescent label. The resulting combinations are called luminescing memories with matrices. When used in SPA formats they are referred to as scintillating matrices with memories and when used in non-radioactive energy transfer formats [such as HTRF] they are referred to as fluorescing memories with matrices.

The data storage device or memory is programmed with or encoded with information that identifies molecules or biological particles, either by their process of preparation, their identity, their batch number, category, physical or chemical properties, combinations of any of such information, or other such identifying information. The molecules or biological particles are in physical contact, direct or indirect, or in proximity with the matrix, which in turn is in physical contact or in the proximity of the recording device that contains the data storage memory. The molecule or biological particle may also be associated, such that a molecule or biological particle that had been linked to or in proximity with a matrix with memory may be identified [i.e., although the matrix particle and biological particle or molecule are not linked or in proximity, the identify of the matrix that had been linked to the molecule or particle is known]. Typically, the matrix is on the surface of the recording device and the molecules and biological particles are in physical contact with the matrix material. In certain embodiments, the memory device may be linked to or in proximity to more than one matrix particle.

The data storage device or memory can also be programmed by virtue of a reaction in proximity to or in the vicinity of the matrix with memory. In particular, the recording devices include memories and also additional components that detect occurrence of external events or to monitor the status of external parameters, such as EM emissions, changes in temperature or pH, ion concentrations and other such solution parameters. For example, recording devices include memories and may also include a photodectector that can detect the occurrence of fluorescence or other optical emission. Coupling this emission with an amplifier and providing a voltage to permit data storage in the matrix with memory during the reaction by way of, for example an RF signal transmitted to and received by an antenna/rectifier combination within the data storage device or providing voltage sufficient to write to memory from a battery [see, e.g., U.S. Patent No. U.S. Patent No. 5,350,645 and U.S. Patent No. 5,089,877], permits occurrence of the emission to be recorded in the memory.

The recording device [containing the memory] is associated with the memory. Typically, the recording device is coated with at least one layer of material, such as a protective polymer or a glass, including polystyrene, heavy metal-free glass, plastic, ceramic, and may be coated with more than one layer of this and other materials. It must be treated to render it suitable for linking molecules or biological particles when it is used as a support. For example, it may be coated with a ceramic or glass that is suitably derivatized and then coated with or linked to the matrix material. Alternatively, the glass or ceramic or other coating may serve as the matrix. In other embodiments the recording device and the matrix material are in proximity, such as in a container of a size approximately that of the device and matrix material. In yet other embodiments the recording device and matrix material are associated, such that the molecule or biological particle that was linked to the matrix or that was in proximity thereto may be identified.

The matrix combinations [the matrices with memories], thus, contain a matrix material, typically in particulate form, in physical contact with a tiny device containing one or more remotely programmable data storage units [memories]. Contact can be effected by placing the recording device with memory on or in the matrix material or in a solution that is in contact with the matrix material or by linking the device, either by direct or indirect covalent or non-covalent interactions, chemical linkages or by other interactions, to the matrix. Alternatively, matrices with memories carry a code, such as a bar code, preferablly a two-dimensional bar code, on typically one surface and the memory is remote, such as a memory in a computer or any written record by which the code can be deciphered and information stored and associated therewith.

For example, when the memories are proximate to the matrix, contact can be effected chemically, by chemically coupling the recording device with memory to the matrix, or physically by coating the recording device with the matrix material or another material, by physically inserting or encasing the device in the matrix material, by placing the device onto the matrix or by any other means by which the device can be placed in contact with or in proximity to the matrix material. The contact may be direct or indirect via linkers. The contact may be effected by absorption or adsorption.

Since matrix materials have many known uses in conjunction with molecules and biological particles, there are a multitude of methods known to artisans of skill in this art for linking, joining or physically contacting the molecule or biological particle with the matrix material. In some embodiments, the recording device with data storage unit is placed in a solution or suspension of the molecule or biological particle of interest. In some of such instances, the container, such as the microtiter plate or test tube or other vial, is the matrix material. The recording device is placed in or on the matrix or is embedded, encased or dipped in the matrix material or otherwise placed in proximity by enclosing the device and matrix material in a

container, preferably a semi-rigid or rigid container, [MICROKAN™] fabricated from, preferably, porous material, such as polytetrafluoroethylene [marketed TEFLON® (Trademark, E. I. DuPont)] or polypropylene prepared with pores, that is inert to the reaction of interest and that have pores of size permeable to desired components of the reaction medium.

More than one data storage device may be in proximity to or contact with a matrix particle, or more than one matrix particle may be in contact with one device. For example, microplates, such as microtiter plates or other such high density format [i.e. 96, 384 1536 or more wells per plate, such as those available from Nunc, Naperville, IL, Costar, Cambridge MA, and Millipore, Bedford, MA] with the recording device containing the data storage unit [remotely programmable memory] embedded in each well or vials [typically with a 1 ml or smaller capacity] with an embedded recording device may be manufactured.

In a preferred embodiment, the recording device is a semiconductor that is approximately about 10 mm-20 mm or less in its largest dimension and the matrix material is a particle, such as a polystyrene bead or encases the device or is coated on material encasing the device. In one embodiment, the device and a plurality of particles, referred to as "beads", typically about 1 mg to about 50 mg, but larger size vessels and amounts up to 1000 mg, preferably 50 to about 200 mg, are sealed in chemically inert porous, preferably relatively rigid supports, such as polypropylene or teflon formed so that it has pores of a selected size that excludes the particles but permits passage of the external medium. For example, a single device and a plurality of particles may be sealed in a porous or semi-permeable inert material to produce a microvessel [such as the MICROKAN™] such as a TEFLON® [polytetrafluoroethylene] or polypropylene or membrane that is permeable to the components of the medium, or they may be contained in a small closable container that has at least one dimension that is porous or is a semi-permeable tube. Typically such tube, which preferably has an end

that can be opened and sealed or closed tightly. These microvessels preferably have a volume of about 100-500 mm^3, but can have smaller or larger volumes, such as greater than 500 mm^3 [or 1000 mm^3] at least sufficient to contain at least 200 mg of matrix particles, such as about 500-3000 mm^3, such as 1000-2000 or 1000 to 1500, with preferred dimensions of about 1-10 mm in diameter and 5 to 20 mm in height, more preferably about 5 mm by 15 mm, or larger, such as about 1-6 cm by 1-6 cm. The porous wall should be non-collapsible with a pore size in the range of 70 μm to about 100 μm, but can be selected to be semi-permeable for selected components of the medium in which the microvessel is placed. The preferred geometry of these combinations is cylindrical. These porous microvessels may be sealed by heat or may be designed to snap or otherwise close. In some embodiments they are designed to be reused. In other embodiments, the microvessel MICROKAN™ with closures may be made out of non-porous material, such as a tube in the conical shape or other geometry.

Such vessels thus are preferably relatively rigid containers with mesh side walls. Typicially, a single compound is synthesized in each one, and each one contains a unique memory with encoded information or a read/write memroy and are designed to be loaded with solid phase resin. Syntheses takes place by allowing reagents to flow through the outer mesh walls. The preferred embodiment has a volume of about 330 μl of which approximately 200 μl is available for resin with the remainder of the space occupied by the electromagnetic tag. Typically about 30 mg of most commercial resins can be loaded into this volume leaving enough space available for the resin to swell and still remain loose within the walls.

Also provided herein, are tubular or hollow devices [or other geometry] is which the recording device is enclosed in a solid polymer, such as a polypropylene or teflon, which is then radiation grafted with selected monomers or otherwise treated to produce a surface suitable for chemical

synthesis and linkage of molecules. These tubular devices may be sealed or open and the recording device may be held inside by friction or sealed therein. These tubular devices (or other geometry) MICROTUBE™ microreactors may contain a recording device or may include a code engraved, such as by a laser, or otherwise imprinted on the surface. The tubular devices are preferably TEFLON® [polytetrafluoroethylene (PFTE)], polyethylene, high density polyethylene, polypropylene, polystyrene, polyester, ceramic, composites of any of these materials and other such materials. A method for radiation grafting of monmers to PFTE is provided herein. These devices may also be formed from a ball with a screw cap [MICROBALLS®].

These types of memories with matrices are polypropylene or fluoropolymer tubes with a radiation grafted functionalized polystyrene surface that completely enclose a selected memory, such as an RF tag. Syntheses are performed on the functionalized polystyrene surface These devices solid phase chemistry without the need to load solid phase resins.

Other devices of interest, are polypropylene or TEFLON supports, generally about 5-10 mm in the largest dimension, and preferably a cube or other such shape, that are marked with a code, and tracked using a remote memory. These microvessels can be marked with a code, such as a bar code, alphanumeric code or other mark, for identification, particularly in embodiments in which the memory is not in proximity to the matrix, but is remote therefrom and used to store information regarding each coded vessel.

The combination of matrix with memory is used by contacting it with, linking it to, or placing it in proximity with a molecule, such as an intermediate in the synthesis of the compounds of Formula (I) provided herein, and/or the complete end-product compounds of Formula (I) prepared according to the methods described herein to produce a second combination

of a matrix with memory and a molecule. A combination of matrix with memory may also be used by contacting it with, linking it to, or placing it in proximity with a biological particle, such as a virus or phage particle, a bacterium or a cell. In certain instances, such combinations of matrix with memory or combination of matrix with memory and molecule or biological particle may be prepared when used or may be prepared before use and packaged or stored as such for futures use. The matrix with memory when linked or proximate to a molecule or biological particle is herein referred to as a microreactor.

The recording device containing the data storage unit(s) with remotely programmable memory, includes, in addition to the remotely programmable memory, means for receiving information for storage in the memory and for retrieving information stored in the memory. Such means is typically an antenna, which also serves to provide power in a passive device when combined with a rectifier circuit to convert received energy, such as RF or microwave, into voltage, that can be tuned to a desired electromagnetic frequency to program the memory. Power for operation of the recording device may also be provided by a battery attached directly to the recording device, to create an active device, or by other power sources, including light and chemical reactions, including biological reactions, that generate energy.

Preferred frequencies are any that do not substantially alter the molecular and biological interactions of interest, such as those that are not substantially absorbed by the molecules or biological particles linked to the matrix or in proximity of the matrix, and that do not alter the support properties of the matrix. Radio frequencies and microwave frequencies are presently preferred, but other frequencies, such as radar, or optical lasers will be used, as long as the selected frequency or optical laser does not interfere with the interactions of the molecules or biological particles of interest. Thus, information in the form of data points corresponding to such

information is stored in and retrieved from the data storage device by application of a selected electromagnetic radiation frequency, which preferably is selected to avoid interference from any background electromagnetic radiation.

The preferred miniature recording device for use in the combinations herein is a single substrate of a size preferably less than about 10 to 20 mm^3 [meaning up to about 10-20 mm in each dimension or 10-20 mm in its largest dimension], that includes a remotely programmable data storage unit(s) [memory], preferably a non-volatile memory, and an antenna for receiving or transmitting an electromagnetic signal [and in some embodiments for supplying power in passive devices when combined with a rectifier circuit] preferably a radio frequency signal; the antenna, rectifier circuit, memory and other components are preferably integrated onto a single substrate, thereby minimizing the size of the device. An active device, i.e., one that does not rely on external sources for providing voltage for operation of the memory, may include a battery for power, with the battery attached to the substrate, preferably on the surface of the substrate. Vias through the substrate can then provide conduction paths from the battery to the circuitry on the substrate. The device is rapidly or substantially instantaneously programmable, preferably in less than 5 seconds, more preferably in about 1 second, and more preferably in about 50 to 100 milliseconds or less, and most preferably in about 1 millisecond or less. In a passive device that relies upon external transmissions to generate sufficient voltage to operate, write to and read from an electronic recording device, the preferred memory is non-volatile, permanent, and relies on antifuse-based architecture or flash memory. Other memories, such as electrically programmable erasable read only memories [EEPROMs] based upon other architectures also can be used in passive devices. In active recording devices that have batteries to assure continuous power availability, a broader range of memory devices may be used in addition to those identified

above. These memory devices include dynamic random access memories [DRAMS, which refer to semiconductor volatile memory devices that allow random input/output of stored information; see, e.g., U.S. Patent Nos. 5,453,633, 5,451,896, 5,442,584, 5,442,212 and 5,440,511], that permit higher density memories, and EEPROMs.

Containers, such as vials, tubes, microtiter plates, reagent bottles, sample and collection vials, autosampler carousels, HPLC columns and other chromatography columns, such as GC columns, electrophoresis and capillary electrophoresis equipment, plate readers, reagent carriers, fraction collectors, capsules and the like, which are in contact with a recording device that includes a data storage unit with programmable memory or include an optical memory, such as a 3-D optical memory incorporated into the material or attached to the container or instrument, and also include the compounds hereinare also provided. The memories may also be used incombination with instruments, which are in contact with a recording device that includes a data storage unit with programmable memory are also provided. The container is typically of a size used in immunoassays or hybridization reactions, including, but not limited to HPLC, gas chromatographs (GC), mass spectrometers (MS), NMR instruments, GC-MS, stir bars ,spectrometers, including fluorimeters, luminometers, and capillary electrophoresis and electrophoresis instruments and tubes and plates used therefor. Thus, an entire laboratory may be augmented with memories linked to or proximate to every container, instrument, and device, from reagent bottle to collected fraction, used in a particular protocol, whereby a sample, i.e., the compounds of formula (I), may be tracked.

Methods for identifiably tagging molecules, such as the compounds of Formula (I) and/or intermediates in the synthesis thereof, or biological particles are provided. Such tagging is effected by placing the molecules or biological particles of interest in proximity with the recording device or with the matrix with memory, and programming or encoding the identity of the

molecule or synthetic history of the molecules or batch number or other identifying information into the memory. Programming is preferably effected during each step of the synthetic protocol. The thus identified molecule or biological particle is then used in the reaction or assay of interest and tracked by virtue of its linkage to the matrix with memory, its proximity to the matrix with memory or its having been linked or in proximity to the matrix [i.e., its association with], which can be queried at will to identify the molecule or biological particle.

The tagging and/or reaction or assay protocols may be automated. Automation may use robotics [see, U.S. Patent No. 5,463,564, which provides an automated iterative method of drug design]. The automated protocols include sorting devices, that permit the directed sorting of the memories with matrices during each step of the synthetic protocol and then before, during and after the assaying protocols. The sorting devices provide the means to read each memory with combination and direct it to the next step in any synthetic or screening protocol. Sorting methods and devices are described herein and also in copending, commonly owned applications, such as U.S. application Serial No. 08/826,253.

In particular, methods for tagging, tracking and sorting constituent members of combinatorial libraries containing compounds of Formula (I) are provided. These methods involve electromagnetically tagging the constituent members of the library by contacting the molecules or bringing such molecules into proximity with a matrix with memory and programming the memory with retrievable information from which the identity, synthesis history, batch number or other identifying information can be retrieved. The contact is effected by coating, completely or in part, the recording device with memory with the matrix, or embedding the recording device with memory within the matrix, and then linking, directly or via linkers, the synthetic intermediate and/or end-product compound to the matrix support. The memories can be coated with a protective coating, such as a glass or

silicon, which can be readily derivatized for chemical linkage or coupling to the matrix material. In other embodiments, the memories can be coated with matrix, such as for example dipping the memory into the polymer prior to polymerization, and allowing the polymer to polymerize on the surface of the memory. Alternatively, the contact is effected by bringing and maintaining the synthetic intermediate and/or end-product compound, preferrably linked to a matrix support, into proximity to the recording device by enclosing the device and matrix material in a microvessel.

In other embodiments, the memory is part of the container that *contains the sample or is part of the instrument tracking the compounds.* As a sample is moved, for example, from container to container or from instrument to container to a plate, the information from one memory is transferred by reading one memory and writing to the next so the identity of the contents is tracked as it is processed. Such movement and tracking can be automated.

If the matrices are used for the synthesis of the constituent molecules, the memory of each particle is addressed and the identity of the added component is encoded in the memory at [before, during, or preferably after] each step in the synthesis. At the end of the synthesis, the memory contains a retrievable record of all of the constituents of the resulting molecule, or following cleavage from the support in an assay or for screening or other such application. If the molecule is cleaved from the support with memory, the memory must remain in proximity to the molecule or must in some manner be traceable [i.e., associated with] to the molecule.

In preferred embodiments, the matrix with memory with linked molecules are mixed and reacted with a sample according to a screening or assay protocol, and those that react are identified. The identity of reacted molecules can then be ascertained by remotely retrieving the information stored in the memory and decoding it to identify the linked molecules.

Synthesis and screening may be effected on a single platform, and methods herein (and also those described in copending applications (see, e.g., copending commonly owned U.S. application Serial No. 08/826,253), to sort and process the memory with matrix microreactors and linked compounds.

In the methods provided herein for tagging constituent members of libraries containing compounds of Formula (I), the matrices are used for the synthesis of each compound, the memory associated with each distinct "R" group substituent (or building block) is addressed, and the identity of the incorporated "R" group molecule (or building block) is encoded in the memory at [before, during or after] each step in the synthesis. At the end of the synthesis, the memory contains a retrievable record of all of the R group substituents (or building blocks) of the resulting compound, which can then be used, either linked to the support, or preferably following cleavage from the support in screeninng assays and in assays to evaluate the properties of the compound or other such application. If the compound is cleaved from the support with memory, the memory must remain in proximity to the compound or must in some manner be traceable [i.e., associated with] to the compound. Such synthetic, cleaving and sorting steps may be automated (see, U.S. application Serial No. 08/826,253).

The matrices with memory with linked compounds of Formula (I) may be mixed and contacted with a sample according to a screening or assay protocol, and those that induce a desired result are isolated. The identity of the isolated molecule can then be ascertained by remotely retrieving the information stored in the memory and decoding it to identify the linked molecules.

In addition, preferred methods for preparing compounds of Formula (I) incorporate the above-described methods for identifiably tagging the compounds and/or intermediates in the synthesis thereof. As a result, distinct compounds of Formula (I) possessing specific R group substitutents

are uniquely tagged during such synthesis. These methods may involve coating, completely or in part, the recording device with memory with the matrix, or embedding the recording device with memory within the matrix, and then linking, directly or via linkers, the synthetic intermediate and/or end-product compound to the matrix support. Alternatively, the synthetic intermediate and/or end-product compound, linked to a matrix support, is maintained in proximity to the recording device with memory by enclosing the device and matrix material in a microvessel.

In the preferred methods provided herein for preparing compounds of Formula (I), the matrices are used for the synthesis of each compound, the memory associated with each distinct R group substituent is addressed, and the identity of the incorporated R group molecule is encoded in the memory at [before, during, or after] each step in the synthesis. At the end of the synthesis, the memory contains a retrievable record of all of the R group substituents of the resulting compound, which can then be used, preferably following cleavage from the support and any further purification that may be required, in assays to evaluate the properties of the compound or as antitumor agents or other such application.

Compositions containing combinations of matrices with memories and molecules (i.e., compounds of Formula (I) and/or synthetic intermediates thereof) are also provided. In particular, coded or electronically tagged libraries of compounds of Formula (I) are provided. Particulate matrices, such as polystyrene beads, with attached memories, or polypropylene tubes with memories embedded therein, and continuous matrices, such as microtiter plates or slabs or polymer, with a plurality of embedded or attached memories are provided.

These combinations of matrices with memories and molecules may be used in any application in which support-bound molecules are used. Such applications include, but are not limited to diagnostics, such as

immunoassays, drug evaluation and screening assays, combinatorial chemistry protocols and other such uses.

Systems and methods for recording and reading or retrieving the information in the data storage devices regarding the identity or synthesis of the compounds are also provided. The systems for recording and reading data include: a host computer or other encoder/decoder instrument having a memory for storing data relating to the identity or synthesis of the compounds, and a transmitter means for receiving a data signal and generating a signal for transmitting a data signal; and a recording device that includes a remotely programmable, preferably non-volatile, memory and transmitter means for receiving a data signal and generating at least a transmitted signal and for providing a write signal to the memory in the recording device. The host computer stores transmitted signals from the memories with matrices, and decodes the transmitted information. The systems also include a means for sorting the matrix with memory combinations by reading the encoded information and directing them accordingly.

In particular, the systems include means for writing to and reading from the memory device to store and identify each of the indicators that identify or track the compounds of Formula (I) and/or synthetic intermediates thereof. The systems additionally include the matrix material in physical contact with or proximate to the recording device, and may also include a device for separating matrix particles with memory so that each particle or memory can be separately programmed.

Methods for tagging molecules (i.e., compounds of Formula (I) and synthetic intermediates thereof) by contacting, either directly or indirectly, a molecule with a recording device; transmitting from a host computer or decoder/encoder instrument to the device electromagnetic radiation representative of a data signal corresponding to an indicator that either specifies one of a series of synthetic steps or the identity or other

information for identification of the molecule, whereby the data point representing the indicator is written into the memory, are provided.

Methods for reading identifying information from recording devices linked to or in contact with or in proximity to or that had been in contact with or proximity to a electromagnetically tagged molecule are provided. These methods include the step of exposing the recording device containing the memory in which the data are stored to electromagnetic radiation [EM]; and transmitting to a host computer or decoder/encoder instrument an indicator representative of the identity of a molecule or identification of the molecule linked to, in proximity to or associated with the recording device.

Of particular interest herein, are multiprotocol applications [such as multiplexed assays or coupled synthetic and assay protocols] in which the matrices with memories are used in a series [more than one] of reactions, particularly whereby compounds of Formula (I) are synthesized, a series [more than one] of assays, such as assays described herein for evaluating the growth inhibitory properties of the compounds, and/or a series of one or more reactions and one or more assays, typically on a single platform or coupled via automated analysis instrumentation.

As a result synthesis is coupled to screening, including compound identification and analysis, where needed. As noted above, where sample is transferred, for example, from vial or tubes to plates, etc., the vials, plates, reagent bottles and columns and other items used in drug discovery or for collecting and analyzing samples, screening and analysis equipment and instrumentation include memory, such as an RF tag, optical memory, such as a 3-D optical memory, or 2-D opical bar code. As a sample is sythesized or obtained and processed, the information is transfered from one memory to the next, thereby providing a means to track the sample and identity from synthesis to screening to analysis.

DESCRIPTION OF THE DRAWINGS

Understanding of the subject matter provided herein will be facilitated by consideration of the detailed description of the preferred embodiments in conjunction with the accompanying drawings, in which like reference numerals refer to like parts and in which:

FIGURE 1 depicts combinatorial synthesis of chemical libraries on matrix supports with memories. A, B, C . . . represent the chemical building blocks; a, b, c . . . represent the codes stored in memory that correspond to each of A, B, C, . . ., respectively. $S_{a,}$, S_b, S_c... represent respective signals sent to memory. Alternatively, the matrix supports are OMDs [optical memory devices] that are encoded with symbology associated with information stored in a remote memory, such as a computer. The symbology may be precoded or encoded prior to or during sythesis.

FIGURE 2 depicts combinatorial synthesis of peptides on a matrix with memory. Each amino acid has a corresponding code, a,b, c ... , in the matrix memory, and L represents a Linker between the memory device and the pharmacophore. Again as in FIGURE 1, the matrix supports may be engraved with a code or symbology associated with information stored in a remote memory.

FIGURE 3 depicts combinatorial synthesis of oligonucleotides on matrix supports with memories. A, G, T and C represent nucleotides, and a, g, t, and c represent the electronic codes stored in memory that correspond to each of A, G T and C,, respectively. The phosphoramidite method of oligonucleotide synthesis is performed by methods known to those of skill in the art [see, e.g., Brown et al. (1991) "Modern machine-aided methods of oligodeoxyribonucleotide synthesis" in Oligonucleotides Analogues EDITOR: Eckstein, Fritz (Ed), IRL, Oxford, UK., pp. 1-24, esp. pp. 4-7]. As

in FIGURES 1 and 2, the matrix may alternatively, or additionally, have symbology engraved thereon.

FIGURE 4 depicts generation of a chemical library, such as a library of organic molecules, in which R_1, R_2, R_3 are substituents on selected molecule, such as a pharmacophore monomer, each identified with a different signal, depicted as 1, 2, or 3, from the classes S_1, S_2, S_3, respectively. The circle represents an organic pharmacophore. If R_1-R_3 are the same, and selected from among the same 50 choices, then the complete library contains 50^3 = 125,000 members. If R_1-R_3 selected from among different sets of choices, then the resulting library has correspondingly more members. Each optical memory device can be encoded with information that represents the R_n added and class $[S_n]$ thereby providing a unique code for each library member. As in FIGURES 1-3, the matrix may be engraved with symbology, such as a two-dimensional bar code.

FIGURE 5 is a block diagram of the data storage means and supporting electrical components of a preferred embodiment.

FIGURE 6 is a diagrammatic view of the memory array within the recording device, and the corresponding data stored in the host computer memory.

FIGURE 7 is an illustration of an exemplary apparatus for separating the matrix particles with memories for individual exposure to an EM signal.

FIGURE 8 is an illustration of a second exemplary embodiment of an apparatus for separating matrix particles for individual exposure to an optical signal.

FIGURE 9 is a diagrammatic view of the memory array within the recording device, the corresponding data stored in the host computer memory, and included photodetector with amplifier and gating transistor.

FIGURE 10 is a scheme for the synthesis of the 8 member RF encoded combinatorial decameric peptide library described in EXAMPLE 4. All couplings were carried out in DMF at ambient temperature for 1 h [two couplings per amino acid], using PyBOP and EDIA or DIEA. Deprotection conditions: 20% piperidine in DMF, ambient temperature, 30 min; Cleavage conditions: 1,2-ethanedithiol:thioanisole:water:phenol:trifluoroacetic acid [1.5:3:3:4.5:88, w/w], ambient temperature, 1.5 h.

FIGURE 11 is a side elevation of a preferred embodiment of a microvessel.

FIGURE 12 is a sectional view, with portions cut away, taken along line 12-12 of FIGURE 11.

FIGURE 13 is a sectional view taken along line 13-13 of FIGURE 12.

FIGURE 14 is a perspective view of an alternative embodiment of a microvessel, with the end cap separated.

FIGURE 15 is a side elevation view of the microvessel of FIGURE 14, with a portion cut away.

FIGURE 16 is a sectional view taken along line 16-16 of FIGURE 15.

FIGURE 17 is a perspective view of an exemplary write/read station.

FIGURE 18 is a flow diagram of the operation of the system of FIGURE 17.

FIGURE 19 shows fluorescent solid supports: application in solid phase synthesis of direct SPA.

FIGURE 20 Coded macro "beads" for efficient combinatorial synthesis.

FIGURE 21 Show the preparation and use of a tubular microvessel in which the container is radiation grafted with monomers or otherwise activated for use as a support matrix.

FIGURE 22 is a perspective view of a first embodiment of an optical memory device;

FIGURE 23 is an exploded perspective view of a second embodiment of the optical memory device;

FIGURE 24 is a diagrammatic view of the optical write and read for the optical memory devices;

FIGURE 25 is a side elevation of a third embodiment of the optical memory device;

FIGURE 26 is a side elevation of a fourth embodiment of the optical memory device;

FIGURE 27 is a side elevation of a fifth embodiment of the optical memory device;

FIGURE 28 is a front elevation of a sixth embodiment of the optical memory device;

FIGURE 29 is a front elevation of a seventh embodiment of the optical memory device;

FIGURE 30 is a front elevation of an eighth embodiment of the optical memory device;

FIGURE 31 is a flow diagram of the image processing sequence for a two-dimensional bar code on an optical memory device; and

FIGURE 32 is a diagrammatic view of an exemplary handling system for feeding, reading and distributing the optical memory devices.

FIGURES 33 depict the OMDs with optical symbology provided herein. FIGURE 33A illustrates an exemplary OMD. FIGURE 33B depicts a close-up of a 2-D laser etched bar codethat is read by the software described herein that reads the code in two dimensions, horizontally and vertically simultaneously using a camera and pattern recognition software described herein. With resference to the Examplified embodiment [see EXAMPLES], the code in this figure is 0409AA55AA550409. The blacked out and whitened squares represent data units. Etching of the entire 2-D bar code by a CO_2 laser can be accomplished with a resolution below about 0.5 mm. FIGURE 33C depicts a split and poool combinatorial synthesis

protocol using the OMDs and directed sorting. A, B and C represent building blocks, and the numbers above each OMD represent a 2-D optical bar code [single digits are used merely for exemplification]. FIGURE 33D depicts synthesis of a 3 x 3 x 3 oligonucleotide library using the OMDs and directed sorting [reaction conditions are described in the EXAMPLES; DMT-X_2, X_3 or X_4 is 5'-O-DMT-2'deoxyadenosine-3'-O-phosphoramidite, 5'-O-DMT-2'deoxycytidine-3'-O-phosphoramidite, 5'-O-DMT-2'deoxyguanosine-3'-O-phosphoramidite; B_2, B_3 or B_4 is adenine, cytosine or guanine. FIGURE 33E depicts an oligonucleotide hexamer library using the optical memory device. Each B^n refers to a nucleoside base, and the resulting library, where n=6, will contain 4096 unique members [4^6 =4096].

FIGURE 34 depicts a protocol for radiation grafting of polymers to the inert surfaces to render them suitable for use as matrices. FIGURE 34A exemplifies the grafting of a polymer to a tube containing an RF tag, linkage of scintillant to the surface, organic synthesis and then use of the resulting compound linked to the support in an assay. Thus, all steps are performed on the same platform. FIGURE 34B also exemplifies a single platform protocol. FIGURE 34C depicts the preparation of a tubular devices in which the matrix is the radiation grafted PTFE and the memory is a transponder, such as the BMDS transponder or IDTAG™ transponder [such as a MICROTUBE], described herein; FIGURE 34D depicts a small chip [2 mm x 2 mm x 0.1 mm] encased in a radiation grafted polyprolene or teflon ball [ball or bead or other such geometry] with a screw cap [such as a MICROBALL or MICROBEAD].

FIGURE 35 exemplifies the general reaction scheme for synthesis of the tyrphostin analogs exemplified where the microreator is a MICROTUBE® microreator with an electromagnetic tag inside;

FIGURE 36 exemplifies synthesis of a specific compound according to the scheme set forth in FIGURE 35, where R^3 is benzoyl, R^2 is methylphenyl,

R^1 is fluorophenyl, and includes the protocol for attaching the linker to the solid support MICROTUBE® microreactor;

FIGURE 35 sets forth exemplary groups for BB1, BB2 and BB3 for a library containing 432 members that has been synthesized;

FIGURE 38 sets forth another set of exemplary groups for BB1, BB2 and BB3 for a library containing 432 members that has been synthesized;

FIGURES 39A-C set forth sets of building blocks for a ~10K member library (note that the R group in FIGURE 39B is any group, and is preferably any of the groups set forth for R^4 or any of the other substituents set forth for R^2);

FIGURE 40 sets for exemplary compounds that were synthesized using the building block set forth in FIGURE 37 or 38, where the reference letters, such as a4, refer to the designation for each building block in FIGURE 37 or 38 (e.g., for a4 BB1 is "a" in FIGURE 37 and BB2 is "4" in FIGURE 37);

FIGURE 41 shows a summary of the conclusions from structure activity relationships (SAR) regarding the requisites for activity based on data using the PTK assay provided herein;

FIGURE 42 shows the results of assays for PTK inhibitory activity and toxicity of some exemplary compounds;

FIGURE 43 is a perspective view of a monolithic identification tag with the antenna formed on the substrate; and

FIGURE 44 is a plan view of the monolithic identification tag as shown in FIGURE 43, showing generally the outline of the circuitry on the substrate, and the formation of the antenna on encircling that circuitry.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

OUTLINE

I. **Definitions**

II. **Combinations of matrices with memory and tyrphostin-based compounds, screening methods, pharmaceutical compositions**

A. Tyrosine kinase inhibitors as anti-proliferative agents

B. Combinations of tyrphositin-based compounds and matrices with memories and libraries containing such compounds

1. Compounds

2. Libraries

3. Practice of the method of preparation of a tyrphostin-based compound library using matrices with memories

a. Preparation of microreactors

(1) Microreactors

(2) Linkers

b. Libraries

c. General synthetic scheme

d. Exemplary embodiment

C. Screening Assays and selection of tyrphostin analogs

D. Pharmaceutical compositions

1. Compounds of Formula (I)

2. Pharmaceutical compositions containing compounds of Formula (I)

III. Combinations of matrices with memories

A. Matrices

1. Natural matrix support materials

2. Synthetic matrices

3. Immobilization and activation

C. Data storage units with memory

1. Optically and magnetically encoded memory devices

a. Encoded memory devices with two-dimensional bar codes and matrices with optical memories

b. Optically or magnetically programmed devices

c. Three dimensional optical memories

d. 3-D Optical memories and apparatus therefor

I. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. All patents and publications referred

to herein are, unless noted otherwise, incorporated by reference in their entirety.

As used herein, a bar code refers any array optically readable marks of any desired size and shape that are arranged in a reference context or frame of, preferably, although not necessarily, one or more columns and one or more rows. For purposes herein, the bar code refers to any symbology, not necessary "bar" but may include dots, characters or any symbol or symbols.

As used herein, an optical memory device [OMD] refers to a surface that is encoded with a code, preferably the 2-D bar code provided herein. For use herein, such devices include at least two surfaces, one of which is treated or formed from a matrix material treated to render it suitable for use as a support to which molecules or biological particles are linked, such as in chemical syntheses or as supports in assays, and the other that includes a code that can be optically read and then compared with information in a computer or other memory to interpret its meaning.

As used herein, an optical memory refers to the symbology and the surface on which it is engraved or otherwise imprinted. For purposes herein, an optical memory is distinct from optical recording media that may be appropriate for use in the recording devices and combinations herein include, but are not limited to, optical discs, magneto-optical materials, photochromic materials, photoferroelectric materials, and photoconductive electro-optic materials.

As used herein, symbology refers to the code, such as a bar code, that is engraved or imprinted on the OMD. The symbology is any code known or designed by the user. The symbols are associated with information stored in a remote computer or memory or other such device or means. For example, each OMD can be uniquely identified with an encoded symbology. The process steps or additions or manipulations to the

associated molecules or biological particles can be recorded in a remote memory and associated with the code.

As used herein, a matrix refers to any solid or semisolid or insoluble support on which a code is to which the memory device and/or the molecule of interest, the compounds provided herein, linked or contacted. Typically a matrix is a substrate material having a rigid or semi-rigid surface and a portion of its surface adapted for binding or linking or contacting compounds during synthesis and screening. In many embodiments, at least one surface of the substrate will be substantially flat, although in some embodiments it may be desirable to physically separate synthesis regions for different polymers with, for example, wells, raised regions, etched trenches, or other such topology. Matrix materials include any materials that are used as affinity matrices or supports for chemical and biological molecule syntheses and analyses, such as, but are not limited to: polystyrene, polycarbonate, polypropylene, nylon, glass, dextran, chitin, sand, pumice, polytetrafluoroethylene, agarose, polysaccharides, dendrimers, buckyballs, polyacrylamide, Kieselguhr-polyacrlamide non-covalent composite, polystyrene-polyacrylamide covalent composite, polystyrene-PEG [polyethyleneglycol] composite, silicon, rubber, and other materials used as supports for solid phase syntheses, affinity separations and purifications, hybridization reactions, immunoassays and other such applications.

Importantly, in all instances it is understood that a least a portion of a surface of the matrix material is treated or rendered suitable for linking compounds. Such treatment includes derivitization or coating with a suitable polymer, such as by radiation grafting followed by suitable derivatization. The matrix herein may be particulate or may be in the form of a continuous surface, such as a microtiter dish or well, a glass slide, a silicon chip, a nitrocellulose sheet, nylon mesh, or other such materials. When particulate, typically the particles have at least one dimension in the 5-10 mm range or smaller. Such particles, referred collectively herein as

"beads", are often, but not necessarily, spherical. Such reference, however, does not constrain the geometry of the matrix, which may be any shape, including random shapes, needles, fibers, elongated and other such geometries. The "beads" may include additional components, such as magnetic or paramagnetic particles [see, e.g., Dyna beads (Dynal, Oslo, Norway)] for separation using magnets, fluophores and other scintillants, as long as the additional components do not interfere with chemical reactions, data entry or retrieval from the memory.

Significantly, it is noted, however, that many surfaces, such as glass, require modification to render them suitable for use as supports. Any such surface must be treated to render it suitable for chemical syntheses or for adsorption of biological particles. Chemical syntheses require a support that not only has the proper surface characteristics [organic solvent wettability, chemical kinetics], but that also has a high density of functional groups. An untreated glass surface contains only a very small amount [less than 1 nmol/sq. mm] of hydroxy groups. It is also very hydrophilic and not very suitable for reactions in organic media. Therefore, the glass surface has to be modified to achieve high functional group density ($\sim >10$ nmol/mm^2) and proper hydrophobicity. Thus, as used herein, matrix refers to materials that have been so-treated. Therefore, a transponder in which the memory device is encased in a glass capsule for instance is not usable as is, but must be treated, either by coating at least one surface with a polymer, such as by grafting, derivatizing or otherwise activating the surface.

As used herein, scintillants include, 2,5-diphenyloxazole [PPO], anthracene, 2-(4'-tert-butylphenyl)-5-(4"-biphenyl)-1,3,4-oxadiazole [butyl-PBD]; 1-phenyl-3-mesityl-2-pyrazoline [PMP], with or without frequency shifters, such as 1,4,-bis[5-phenyl(oxazolyl)benzene] [POPOP]; *p*-bis-*o*-methylstyrylbenzene [bis-MSB]. Combinations of these fluors, such as PPO and POPOP or PPO and bis-MSB, in suitable solvents, such as benzyltoluene

[see, e.g., U.S. Patent No. 5,410,155], are referred to as scintillation cocktails.

As used herein a luminescent moiety refers to a scintillant or fluophor used in scintillation proximity assays or in non-radioactive energy transfer assays, such as HTRF assays.

As used herein, fluorescent resonance energy transfer [FRET] is an art-recognized term meaning that one fluorophore [the acceptor] can be promoted to an excited electronic state through quantum mechanical coupling with and receipt of energy from an electronically excited second fluorophore [the donor]. This transfer of energy results in a decrease in visible fluorescence emission by the donor and an increase in fluorescent energy emission by the acceptor. Significant energy transfer can only occur when the donor and acceptor are sufficiently closely positioned since the efficiency of energy transfer is highly dependent upon the distance between donor and acceptor fluorophores.

As used herein, matrix particles refer to matrix materials that are in the form of discrete particles. The particles have any shape and dimensions, but typically have at least one dimension that is 100 mm or less, preferably 50 mm or less, more preferably 10 mm or less, and typically have a size that is 100 mm^3 or less, preferably 50 mm^3 or less, more preferably 10 mm^3 or less, and most preferably 1 mm^3 or less. The matrices may also be continuous surfaces, such as microtiter plates [e.g., plates made from polystyrene or polycarbonate or derivatives thereof commercially available from Perkin Elmer Cetus and numerous other sources, and Covalink trays [Nunc], microtiter plate lids or a test tube, such as a 1 ml Eppendorf or similar tube or smaller versions, such as 500 μl, 200 μl or smaller. Matrices that are in the form of containers refers to containers, such as test tubes and microplates and vials that are typically used for solid phase syntheses of combinatorial libraries or as pouches, vessels, bags, and microvessels for screening and diagnostic assays. Thus, a container used for chemical

syntheses refers to a container that typically has a volume of about 1 liter, generally 100 ml, and more often 10 ml or less, 5 ml or less, preferably 1 ml or less, and as small as about 50 μl-500 μl, such as 100 μl, 200 μl or 250 μl. This also refers to multi-well plates, such as microtiter plates [96 well, 384 well, 1536 well or other density format]. Such microtiter plate will typically contain a recording device in, on, or otherwise in contact with in each of a plurality of wells.

As used herein, a matrix with a memory refers to a combination of a matrix with a miniature recording device that stores multiple bits of data by which the matrix may be identified, preferably in a non-volatile memory that can be written to and read from by transmission of electromagnetic radiation from a remote host, such as a computer. By miniature is meant of a size less than about 10-20 mm^3 [or 10-20 mm in the largest dimension]. Preferred memory devices or data storage units are miniature and are preferably smaller than 10-20 mm^3 [i.e. up to 10 to 20 mm in each dimension or 10-20 mm in its largest dimension] dimension, more preferably less than 5 mm^3, most preferably about 1 mm^3 or smaller. Alternatively, the memory may be fabricated as part of the matrix material or may be a chemical or biological-based memory means, such as those described herein, including the rhodopsin based memories and 3-D optical memories based on photochromic materials [see, e.g.,U.S. Patent Nos. 5,268,862, 5,130,362, 5,325,324; see, also, Dvornikov et al. (1996) Opt. Commun. 128:205-210; Dvornikov et al. (1996) Res. Chem. Intermed. 22:115-28; Dvornikov et al. (1994) Proc. SPIE-Int. Soc. Opt. Eng. 2297:447-51; Dvornikov et al. (1994) Mol. Cryst. Liq. Cryst. Sci. Technol., Sect. A 246:379-88; Dvornikov et al. (1994) J. Phys. Chem. 98:6746-52; Ford et al. (1993) Proc. SPIE-Int. Soc. Opt. 2026:604-613; Ford et al. Proc. SPIE-Int. Soc. Opt. Eng. 1853:5-13; Malkin et al. Res. Chem. Intermed. 19:159-89; Dvornikov et al. (1993) Proc. SPIE-Int. Soc. Opt. Eng. 1852:243-52; Dvornikov et al. (1992) Proc. SPIE-Int. Soc. Opt. Eng. 1662:197-204; Prasad et al. (1996) Mater. Res. Soc. Symp. Proc. 413:203-213].

As used herein, a microreactor refers to combinations of matrices with memories with associated, such as linked or proximate, biological particles or molecules. It is produced, for example, when the molecule is linked thereto or synthesized thereon. It is then used in subsequent protocols, such as immunoassays and scintillation proximity assays.

As used herein, a combination herein called a microvessel [e.g., a microvessel such as those designated presently designated a MICROKAN™] refers to a combination in which a single device [or more than one device] and a plurality of particles are sealed in a porous or semi-permeable inert material, such as polytetrafluoroethylene or polypropylene or membrane that is permeable to the components of the medium, but retains the particles and memory, or are sealed in a small closable container that has at least one dimension that is porous or semi-permeable. Typically such microvessels, which preferably have at least one end that can be opened and sealed or closed tightly, has a volume of about 200-500 mm^3, with preferred dimensions of about 1-10 mm in diameter and 5 to 20 mm in height, more preferably about 5 mm by 15 mm. The porous wall should be non-collapsible with a pore size in the range of 70 μM to about 100 μM, but can be selected to be semi-permeable for selected components of the reaction medium.

As used herein, a memory is a data storage unit [or medium] with programmable memory, preferably a non-volatile memory; or alternatively is a symbology on a surface, such as a bar code, whose identity and as for which associate information is stored in a remote memory, such as a computer memory.

As used herein, programming refers to the process by which data or information is entered and stored in a memory. A memory that is programmed is a memory that contains retrievable information.

As used herein, remotely programmable, means that the memory can be programmed without direct physical or electrical contact or can be programmed from a distance, typically at least about 10 mm, although shorter distances may also be used, such as instances in which the

information comes from surface or proximal reactions or from an adjacent memory or in instances, such as embodiments in which the memories are very close to each other, as in microtiter plate wells or in an array.

As used herein, a recording device [or memory device] is an apparatus that includes the data storage unit with programmable memory, and, if necessary, means for receiving information and for transmitting information that has been recorded. It includes any means needed or used for writing to and reading from the memory. The recording devices intended for use herein, are miniature devices that preferably are smaller than 10-20 mm^3 [or 10-20 mm in their largest dimension], and more preferably are closer in size to 1 mm^3 or smaller that contain at least one such memory and means for receiving and transmitting data to and from the memory. The data storage device also includes optical memories, such as bar codes, on devices such as OMDs.

As used herein, a data storage unit with programmable memory includes any data storage means having the ability to record multiple discrete bits of data, which discrete bits of data may be individually accessed [read] after one or more recording operations. Thus, a matrix with memory is a combination of a matrix material with a data storage unit.

As used herein, programmable means capable of storing unique data points. Addressable means having unique locations that may be selected for storing the unique data points.

As used herein, reaction verifying and reaction detecting are interchangeable and refer to the combination that also includes elements that detect occurrence of a reaction or event of interest between the associated molecule or biological particle and its environment [i.e., detects occurrence of a reaction, such as ligand binding, by virtue of emission of EM upon reaction or a change in pH or temperature or other parameter].

As used herein, a host computer or decoder/encoder instrument is an instrument that has been programmed with or includes information [i.e., a key] specifying the code used to encode the memory devices. This instrument or one linked thereto transmits the information and signals to the recording device and it, or another instrument, receives the information transmitted from the recording device upon receipt of the appropriate signal. This instrument thus creates the appropriate signal to transmit to the recording device and can interpret transmitted signals. For example, if a "1" is stored at position 1,1 in the memory of the recording device means, upon receipt of this information, this instrument or computer can determine that this means the linked molecule is, for example, a peptide containing alanine at the N-terminus, an organic group, organic molecule, oligonucleotide, or whatever this information has been predetermined to mean. Alternatively, the information sent to and transmitted from the recording device can be encoded into the appropriate form by a person.

As used herein, an electromagnetic tag is a recording device that has a memory that contains unique data points that correspond to information that identifies molecules or biological particles linked to, directly or indirectly, in physical contact with or in proximity [or associated with] to the device. Thus, electromagnetic tagging is the process by which identifying or tracking information is transmitted [by any means and to any recording device memory, including optical and magnetic storage media] to the recording device.

As used herein, proximity means within a very short distance, generally less than 0.5 inch, typically less than 0.2 inches. In particular, stating that the matrix material and memory, or the biological particle or molecule and matrix with memory are in proximity means that, they are at least or at least were in the same reaction vessel or, if the memory is removed from the reaction vessel, the identity of the vessel containing the molecules or biological particles with which the memory was proximate or linked is tracked or otherwise known.

As used herein, associated with means that the memory must remain in proximity to the molecule or biological particle or must in some manner be traceable to the molecule or biological particle. For example, if a molecule is cleaved from the support with memory, the memory must in some manner be identified as having been linked to the cleaved molecule. Thus, a molecule or biological particle that had been linked to or in proximity to a matrix with memory is associated with the matrix or memory if it can be identified by querying the memory.

As used herein, antifuse refers to an electrical device that is initially an open circuit that becomes a closed circuit during programming, thereby providing for non-volatile memory means and, when accompanied by appropriate transceiver and rectification circuitry, permitting remote programming and, hence identification. In practice, an antifuse is a substantially nonconductive structure that is capable of becoming substantially conductive upon application of a predetermined voltage, which exceeds a threshold voltage. An antifuse memory does not require a constant voltage source for refreshing the memory and, therefore, may be incorporated in a passive device. Other memories that may be used include, but are not limited to: EEPROMS, DRAMS and flash memories.

As used herein, flash memory is memory that retains information when power is removed [see, e.g., U.S. Patent No. 5,452,311, U.S. Patent No. 5,452,251 and U.S. Patent No. 5,449,941]. Flash memory can be rewritten by electrically and collectively erasing the stored data, and then by programming.

As used herein, passive device refers to an electrical device which does not have its own voltage source and relies upon a transmitted signal to provide voltage for operation.

As used herein, electromagnetic [EM] radiation refers to radiation understood by skilled artisans to be EM radiation and includes, but is not limited to radio frequency [RF], infrared [IR], visible, ultraviolet [UV], radiation, sonic waves, X-rays, and laser light.

As used herein, information identifying or tracking a biological particle or molecule, refers to any information that identifies the molecule or biological particle, such as, but not limited to the identity particle [i.e. its chemical formula or name], its sequence, its type, its class, its purity, its properties, such as its binding affinity for a particular ligand. Tracking means the ability to follow a molecule or biological particle through synthesis and/or process steps. The memory devices herein store unique indicators that represent any of this information.

As used herein, combinatorial chemistry is a synthetic strategy that produces diverse, usually large, chemical libraries. It is the systematic and repetitive, covalent connection of a set, the basis set, of different monomeric building blocks of varying structure to each other to produce an array of diverse molecules [see, e.g., Gallop et al. (1994) J. Medicinal Chemistry 37:1233-1251]. It also encompasses other chemical modifications, such as cyclizations, eliminations, cleavages, and othe sucht reactions, that are carried in manner that generates permutations and thereby collections of diverse molecules.

As used herein, a biological particle refers to a virus, such as a viral vector or viral capsid with or without packaged nucleic acid, phage, including a phage vector or phage capsid, with or without encapsulated nucleotide acid, a single cell, including eukaryotic and prokaryotic cells or fragments thereof, a liposome or micellar agent or other packaging particle, and other such biological materials.

As used herein, the molecules in the combinations include any molecule, including nucleic acids, amino acids, other biopolymers, and other organic molecules, including peptidomimetics and monomers or polymers of small organic molecular constituents of non-peptidic libraries, that may be identified by the methods here and/or synthesized on matrices with memories as described herein. In particular, molecules refers to the tyrphostin analogs or synthetic intermediates.

As used herein, the term "bio-oligomer" refers to a biopolymer of less than about 100 subunits. A bio-oligomer includes, but is not limited to, a peptide, i.e., containing amino acid subunits, an oligonucleotide, i.e., containing nucleoside subunits, a peptide-oligonucleotide chimera, peptidomimetic, and a polysaccharide.

As used herein, the term "library" refers to a collection of substantially random compounds or biological particles expressing random peptides or proteins or to a collection of diverse compounds. Of particular interest are bio-oligomers, biopolymers, or diverse organic compounds or a set of compounds prepared from monomers based on a selected pharmacophore, in this instance tyrphostin AG490.

As used herein, an analyte is any substance that is analyzed or assayed in the reaction of interest. Thus, analytes include the substrates, products and intermediates in the reaction, as well as the enzymes and cofactors.

As used herein, multianalyte analysis is the ability to measure many analytes in a single specimen or to perform multiple tests from a single specimen. The methods and combinations herein provide means to identify or track individual analytes from among a mixture of such analytes.

As used herein, a fluophore or a fluor is a molecule that readily fluoresces; it is a molecule that emits light following interaction with radiation. The process of fluorescence refers to emission of a photon by a molecule in an excited singlet state. Exemplary fluophores include, but are not limited to Texas Red, acridine, fluorescein, ellipticine, rhodamine, Lissamine rhodamine B, Malachite Green, erythrosin, tetramethylrhodamine, eosin, pyrene, anthracene, methidium, ethydium, phenanthroline, 4-dimethylaminonaphthalene, quinoxaline, 2-dimethylaminonaphthalene, 7-dimethylamino-4-methylcoumarin, 7-dimethylaminocoumarin, 7-hydroxy-4-methylcoumarin, 7-hydroxycoumarin, 7-methoxycoumarin, 7-acetoxycoumarin, 7-diethylamino-3-phenyl-4-methylcoumarin, isoluminol, benzophenone, dansyl, dabsyl, mansyl, sulfo rhodamine, 4-acetamido-4'-

stilbene-2,2'-disulfonic acid disodium salt, 4-benzamido-4'-stilbene-2,2'-disulfonic acid disodium salt. For scintillation assays, combinations of fluors are typically used. A primary fluor that emits light following interaction with radiation and a secondary fluor that shifts the wavelength emitted by the primary fluor to a higher more efficiently detected wavelength.

As used herein, a chromophore is a chemical group that absorbs light at a specific frequency and thereby imparts a detectable color (e.g., detectable spectrophotometrically. Exemplary chromphores include, but are not limited to iron-salicylate complex, indamine dye, INT formazon dye, Hopkins-Cole dye, quinone-imine dye, malachite green, cresol red, diphenylcarbazone, disulphonic acid, Chrome bordeaux B, calmagite, ninhydrin dye, p-nitroanilide, and 4-methoxy-β-naphthylamide.

As used herein, complete coupling means that the coupling reaction is driven substantially to completion despite or regardless of the differences in the coupling rates of individual components of the reaction, such as amino acids In addition, the amino acids, or whatever is being coupled, are coupled to substantially all available coupling sites on the solid phase support so that each solid phase support will contain essentially only one species of peptide.

As used herein, the biological activity or bioactivity of a particular compound includes any activity induced, potentiated or influenced by the compound <u>in vivo</u> or <u>in vitro</u>. It also includes the abilities, such as the ability of certain molecules to bind to particular receptors and to induce [or modulate] a functional response. It may be assessed by <u>in vivo</u> assays or by <u>in vitro</u> assays, such as those exemplified herein.

As used herein, adequately pure or "pure" per se means sufficiently pure for the intended use of the adequately pure compound.

As used herein, substantially pure means sufficiently homogeneous to appear free of readily detectable impurities as determined by standard methods of analysis, such as thin layer chromatography [TLC], mass spectrometry [MS], size exclusion chromatography, gel electrophoresis,

particularly agarose and polyacrylamide gel electrophoresis [PAGE] and high performance liquid chromatography [HPLC], used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical and chemical properties, such as enzymatic and biological activities, of the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound may, however, be a mixture of stereoisomers. In such instances, further purification might increase the specific activity of the compound.

As used herein, biological activity refers to the in vivo activities of a compound or physiological responses that result upon in vivo administration of a compound, composition or other mixture. Biological activity, thus, encompasses therapeutic effects and pharmaceutical activity of such compounds, compositions and mixtures.

As used herein, a prodrug is a compound that, upon in vivo administration, is metabolized or otherwise converted to the biologically, pharmaceutically or therapeutically active form of the compound. To produce a prodrug, the pharmaceutically active compound is modified such that the active compound will be regenerated by metabolic processes. The prodrug may be designed to alter the metabolic stability or the transport characteristics of a drug, to mask side effects or toxicity, to improve the flavor of a drug or to alter other characteristics or properties of a drug. By virtue of knowledge of pharmacodynamic processes and drug metabolism in vivo, those of skill in this art, once a pharmaceutically active compound is known, can design prodrugs of the compound [see, e.g., Nogrady (1985) Medicinal Chemistry A Biochemical Approach, Oxford University Press, New York, pages 388-392].

As used herein, Southern, Northern, Western and dot blot procedures refer to those in which DNA, RNA and protein patterns, respectively, are transferred for example, from agarose gels, polyacrylamide gels or other suitable medium that constricts convective motion of molecules, to

nitrocellulose membranes or other suitable medium for hybridization or antibody or antigen binding are well known to those of skill in this art [see, e.g., Southern (1975) J. Mol. Biol. 98:503-517; Ketner et al. (1976) Proc. Natl. Acad. Sci. U.S.A. 73:1102-1106; Towbin et al. (1979) Proc. Natl. Acad. Sci. U.S.A. 76:4350].

As used herein, a receptor refers to a molecule that has an affinity for a given ligand. Receptors may be naturally-occurring or synthetic molecules. Receptors may also be referred to in the art as anti-ligands. As used herein, both terms, receptor and anti-ligand are interchangeable. Receptors can be used in their unaltered state or as aggregates with other species. Receptors may be attached, covalently or noncovalently, or in physical contact with, to a binding member, either directly or indirectly via a specific binding substance or linker. Examples of receptors, include, but are not limited to: antibodies, cell membrane receptors surface receptors and internalizing receptors, monoclonal antibodies and antisera reactive with specific antigenic determinants [such as on viruses, cells, or other materials], drugs, polynucleotides, nucleic acids, peptides, cofactors, lectins, sugars, polysaccharides, cells, cellular membranes, and organelles.

Examples of receptors and applications using such receptors, include but are not restricted to:

a) enzymes: specific transport proteins or enzymes essential to survival of microorganisms, which could serve as targets for antibiotic [ligand] selection;

b) antibodies: identification of a ligand-binding site on the antibody molecule that combines with the epitope of an antigen of interest may be investigated; determination of a sequence that mimics an antigenic epitope may lead to the development of vaccines of which the immunogen is based on one or more of such sequences or lead to the development of related diagnostic agents or compounds useful in therapeutic treatments such as for auto-immune diseases

c) nucleic acids: identification of ligand, such as protein or RNA, binding sites;

d) catalytic polypeptides: polymers, preferably polypeptides, that are capable of promoting a chemical reaction involving the conversion of one or more reactants to one or more products; such polypeptides generally include a binding site specific for at least one reactant or reaction intermediate and an active functionality proximate to the binding site, in which the functionality is capable of chemically modifying the bound reactant [see, e.g., U.S. Patent No. 5,215,899];

e) hormone receptors: determination of the ligands that bind with high affinity to a receptor is useful in the development of hormone replacement therapies; for example, identification of ligands that bind to such receptors may lead to the development of drugs to control blood pressure; and

f) opiate receptors: determination of ligands that bind to the opiate receptors in the brain is useful in the development of less-addictive replacements for morphine and related drugs.

As used herein, antibody includes antibody fragments, such as Fab fragments, which are composed of a light chain and the variable region of a heavy chain.

As used herein, complementary refers to the topological compatibility or matching together of interacting surfaces of a ligand molecule and its receptor. Thus, the receptor and its ligand can be described as complementary, and furthermore, the contact surface characteristics are complementary to each other.

As used herein, a ligand-receptor pair or complex formed when two macromolecules have combined through molecular recognition to form a complex.

As used herein, an epitope refers to a portion of an antigen molecule that is delineated by the area of interaction with the subclass of receptors known as antibodies.

As used herein, a ligand is a molecule that is specifically recognized by a particular receptor. Examples of ligands, include, but are not limited to, agonists and antagonists for cell membrane receptors, toxins and venoms, viral epitopes, hormones [e.g., steroids], hormone receptors, opiates, peptides, enzymes, enzyme substrates, cofactors, drugs, lectins, sugars, oligonucleotides, nucleic acids, oligosaccharides, proteins, and monoclonal antibodies.

As used herein, a sensor is a device or apparatus that monitors external parameters (i.e., conditions), such as ion concentrations, pH, temperatures. Biosensors are sensors that detect biological species. Sensors encompass devices that rely on electrochemical, optical, biological and other such means to monitor the environment.

As used herein, multiplexing refers to performing a series of synthetic and processing steps and/or assaying steps on the same platform [i.e. solid support or matrix] or coupled together as part of the same automated coupled protocol, including one or more of the following, synthesis, preferably accompanied by writing to the linked memories to identify linked compounds, screening, including using protocols with matrices with memories, and compound identification by querying the memories of matrices associated with the selected compounds. Thus, the platform refers system in which all manipulations are performed. In general it means that several protocols are coupled and performed sequentially or simultaneously.

As used herein, a platform refers to the devices in which on which a reaction or series of reactions is(are) performed.

As used herein a protecting group refers to a material that is chemically bound to a monomer unit that may be removed upon selective exposure to an activator such as electromagnetic radiation and, especially ultraviolet and visible light, or that may be selectively cleaved. Examples of protecting groups include, but are not limited to: those containing nitropiperonyl, pyrenylmethoxy-carbonyl, nitroveratryl,

nitrobenzyl, dimethyl dimethoxybenzyl, 5-bromo-7-nitroindolinyl, o-hydroxy-alpha -methyl cinnamoyl, and 2-oxymethylene anthraquinone.

Also protected amino acids are readily available to those of skill in this art. For example, Fmoc and Boc protected amino acids can be obtained from Fluka, Bachem, Advanced Chemtech, Sigma, Cambridge Research Biochemical, Bachem, or Peninsula Labs or other chemical companies familiar to those who practice this art.

As used herein an effective amount of a compound for treating a particular disease is an amount that is sufficient to ameliorate, or in some manner reduce the symptoms associated with the disease. Such amount may be administered as a single dosage or may be administered according to a regimen, whereby it is effective. The amount may cure the disease but, typically, is administered in order to ameliorate the symptoms of the disease. Typically, repeated administration is required to achieve the desired amelioration of symptoms.

As used herein, an agonist is a compound that potentiates or exhibits a biological activity associated with or possessed by a particular biological molecule..

As used herein, an antagonist is a compound, such as a drug or an antibody, that inhibits a biological or physiological responses. The antagonist may act by interfering with the interaction of a particular molecule with its receptor or by interfering with the physiological response to or bioactivity of an moleucle. Agonist and antagonist activities, collectively referred to as modulatory acitives can be assessed by assays known to those of skill in the art or provided herein.

As used herein, the IC_{50} refers to an amount, concentration or dosage of a particular test compound that achieves a 50% inhibition of a maximal response in an assay [in vitro or in vivo] that measures such response.

As used herein, EC_{50} refers to a dosage, concentration or amount of a particular test compound that elicits a dose-dependent response at 50% of maximal expression of a particular response that is induced, provoked or potentiated by the particular test compound.

As used herein, pharmaceutically acceptable salts, esters or other derivatives of the compounds include any salts, esters or derivatives that may be readily prepared by those of skill in this art using known methods for such derivatization and that produce compounds that may be administered to animals or humans without substantial toxic effects and that either are pharmaceutically active or are prodrugs. For example, hydroxy groups can be esterified or etherified.

As used herein, treatment means any manner in which the symptoms of a conditions, disorder or disease are ameliorated or otherwise beneficially altered. Treatment also encompasses any pharmaceutical use of the compositions herein, such as use as contraceptive agents.

As used herein, amelioration of the symptoms of a particular disorder by administration of a particular pharmaceutical composition refers to any lessening, whether permanent or temporary, lasting or transient that can be attributed to or associated with administration of the composition.

As used herein, acid isostere means a group that is significantly ionized at physiological pH. Examples of suitable acid isosteres include sulfo, phosphono, alkylsulfonylcarbamoyl, tetrazolyl, arylsulfonylcarbamoyl or heteroarylsulfonylcarbamoyl.

As used herein, halo or halide refers to the halogen atoms; F, Cl, Br and I.

As used herein, pseudohalides are compounds that behave substantially similar to halides. Such compounds can be used in the same manner and treated in the same manner as halides (X^-, in which X is a halogen, such as Cl or Br). Pseudohalides include, but are not limited to cyanide, cyanate, thiocyanate, selenocyanate and azide.

As used herein, haloalkyl refers to a loweralkyl radical in which one or more of the hydrogen atoms are replaced by halogen including, but not limited to, chloromethyl, trifluoromethyl, 1-chloro-2-fluoroethyl and the like.

As used herein, alkyl means an aliphatic hydrocarbon group that is a straight or branched chain preferably having about 1 to 12 carbon atoms in the chain. Preferred alkyl groups are loweralkyl groups which are alkyls containing 1 to about 6 carbon atoms in the chain. Branched means that one or more loweralkyl groups such as methyl, ethyl or propyl are attached to a linear alkyl chain. The alkyl group may be unsubstituted or independently substituted by one or more groups, such as, but not limited to: halo, carboxy, formyl, sulfo, sulfino, carbamoyl, amino and imino. Exemplary alkyl groups include methyl, ethyl, propyl, methanoic acid, ethanoic acid, propanoic acid, ethanesulfinic acid and ethane sulfonic acid.

As used herein the term lower describes alkyl, alkenyl and alkynyl groups containing about 6 carbon atoms or fewer. It is also used to describe aryl groups or heteroaryl groups that contain 6 or fewer atoms in the ring. Loweralkyl, lower alkenyl, and lower alkynyl refer to carbon chains having less than about 6 carbons. In preferred embodiments of the compounds provided herein that include alkyl, alkenyl, or alkynyl portions include loweralkyl, lower alkenyl, and lower alkynyl portions.

As used herein, alkenyl means an aliphatic hydrocarbon group containing a carbon-carbon double bond and which may be straight or branched chained having from about 2 to about 10 carbon atoms in the chain. Preferred alkenyl groups have 2 to about 4 carbon atoms in the chain. Branched means that one or more loweralkyl or lower alkenyl groups are attached to a linear alkenyl chain. The alkenyl group may be unsubstituted or independently substituted by one or more groups, such as halo, carboxy, formyl, sulfo, sulfino, carbamoyl, amino and imino. Exemplary alkenyl groups include ethenyl, propenyl, carboxyethenyl, carboxypropenyl, sulfinoethenyl and sulfonoethenyl.

As used herein, alkynyl means an aliphatic hydrocarbon group containing a carbon-carbon triple bond and which may be straight or branched having about 2 to 10 carbon atoms in the chain. Branched means that one or more loweralkyl, alkenyl or alkynyl groups are attached to a linear alkynyl chain. An exemplary alkynyl group is ethynyl.

As used herein, aryl means an aromatic monocyclic or multicyclic hydrocarbon ring system containing from 3 to 15 or 16 carbon atoms, preferably from 5 to 10. Aryl groups include, but are not limited to groups, such as phenyl, substituted phenyl, napthyl, substituted naphthyl, in which the substitunent is loweralkyl, halogen, or lower alkoxy. Preferred aryl groups are lower aryl groups that contain less than 7 carbons in the ring structure. Heteroaryl refers to aromatic rings tha include one or more heteroatoms.

As used herein, the nomenclature alkyl, alkoxy, carbonyl, etc. are used as is generally understood by those of skill in this art. For example, as used herein alkyl refers to saturated carbon chains that contain one or more carbons; the chains may be straight or branched or include cyclic portions or be cyclic.

As used herein, cycloalkyl refers to saturated cyclic carbon chains; cycloalkyenyl and cycloalkynyl refer to cyclic carbon chains that include at least one unsaturated double or triple bond, respectively. The cyclic portions of the carbon chains may include one ring or two or more fused rings.

As used herein, cycloalkenyl means a non-aromatic monocyclic or multicyclic ring system containing a carbon-carbon double bond and having about 3 to about 10 carbon atoms. Exemplary monocyclic cycloalkenyl rings include cyclopentenyl or cyclohexenyl; preferred is cyclohexenyl. An exemplary multicyclic cycloalkenyl ring is norbornylenyl. The cycloalkenyl group may be independently substituted by one or more halo or alkyl.

As used herein, "haloalkyl" refers to a loweralkyl radical in which one or more of the hydrogen atoms are replaced by halogen including, but not limited to, chloromethyl, trifluoromethyl, 1-chloro-2-fluoroethyl and the like.

As used herein, "haloalkoxy" refers to RO- in which R is a haloalkyl group.

As used herein, "carboxamide" refers to groups of formula R_pCONH_2 in which R is selected from alkyl or aryl, preferably loweralkyl or lower aryl and p is 0 or 1.

As used herein, "alkylaminocarbonyl" refers to -C(O)NHR in which R is hydrogen, alkyl, preferably loweralkyl or aryl, preferably lower aryl.

As used herein "dialkylaminocarbonyl" as used herein refers to -C(O)NR'R in which R' and R are independently selected from alkyl or aryl, preferably loweralkyl or loweraryl; "carboxamide" refers to groups of formula NR'COR.

As used herein, "alkoxycarbonyl" as used herein refers to -C(O)OR in which R is alkyl, preferably loweralkyl or aryl, preferably lower aryl.

As used herein, "alkoxy" and "thioalkoxy" refer to RO- and RS-, in which R is alkyl, preferably loweralkyl or aryl, preferably lower aryl.

As used herein, "haloalkoxy" refers to RO- in which R is a haloalkyl group.

As used herein, "aminocarbonyl" refers to $-C(O)NH_2$.

As used herein, "alkylaminocarbonyl" refers to -C(O)NHR in which R is alkyl, preferably loweralkyl or aryl, preferably lower aryl.

As used herein, "alkoxycarbonyl" refers to -C(O)OR in which R is alkyl, preferably loweralkyl.

As used herein, cycloalkyl refers to satured cyclic carbon chains; cycloalkyenyl and cycloalkynyl refer to cyclic carbon chains that include at least one unsaturated triple bond. The cyclic portions of the carbon chains may include one ring or two or more fused rings.

As used herein, alkylenedioxy means an -O-alkyl-O- group in which the alkyl group is as previously described. A replacement analog of

alkylenedioxy means an alkylenedioxy in which one or both of the oxygen atoms is replaced by a similar behaving atom or group of atoms such as, S, N, NH, Se. An exemplary replacement alkylenedioxy group is ethylene-bis(sulfandiyl). Alkylenethioxyoxy is —S-alkyl-O— , —O-alkyl-S— and alkylenedithioxy is —S-alkyl-S—.

As used herein, heteroaryl refers to a monocyclic or multicyclic ring system, preferably of about 5 to about 10 members where one or more, more preferably 1 to 3 of the atoms in the ring system is a heteroatom, that is, an element other than carbon, for example, nitrogen, oxygen and sulfur atoms. The heteroaryl may be optionally substituted with one or more, preferably 1 to 3, substituents, termed aryl group substituents, which are preferably selected from among alkyl, halide, alkoxy, nitro, thioxy, alkylthioxy, haloalky, cyano, amino, hydroxy and haloalkyl. Exemplary heteroaryl groups include, for example, pyrimidinyl, furyl, thienyl, pyridyl, pyrrolyl, N-methylpyrrolyl, quinolinyl and isoquinolinyl. Preferred heteroaryl groups include 5 to 6-membered nitrogen-containing rings, such as pyrmidinyl.

As used herein, heterocyclic refers to a monocyclic or multicyclic ring system, preferably of 3 to 10 members, more preferably 4 to 7 members, even more preferably 5 to 6 members, where one or more, preferably 1 to 3 of the atoms in the ring system is a heteroatom, that is, an element other than carbon, for example, nitrogen, oxygen and sulfur atoms. The heterocycle may be optionally substituted with one or more, preferably 1 to 3 aryl group substituents. Preferred substituents of the heterocyclic group include hydroxy, alkoxy containing 1 to 4 carbon atoms, halo lower alkyl, including trihalomethyl, such as trifluoromethyl, and halogen. As used herein, the term heterocycle may include reference to heteroaryl. Exemplary heterocycles include, for example, pyrrolidinyl, piperidinyl, alkylpiperidinyl, morpholinyl, oxadiazolyl or triazolyl.

As used herein, alkoxycarbonyl means an alkyl-O-CO- group. Exemplary alkoxycarbonyl groups include methoxy- and ethoxycarbonyl.

As used herein, carbamoyl means $-CONH_2$. As with all groups described herein, these groups may be unsubstituted or substituted. Substituted carbamoyl includes groups such as $-CONY^2Y^3$ in which Y^2 and Y^3 are independently hydrogen, alkyl, cyano(loweralkyl), aryalkyl, heteroaralkyl, carboxy(loweralkyl), carboxy(aryl substituted loweralkyl), carboxy(carboxy substituted loweralkyl), carboxy(hydroxy substituted loweralkyl), carboxy(heteroaryl substituted loweralkyl), carbamoyl(loweralkyl), alkoxycarbonyl(loweralkyl) or alkoxycarbonyl(aryl substituted loweralkyl), provided that only one of Y^2 and Y^3 may be hydrogen and when one of Y^2 and Y^3 is carboxy(loweralkyl), carboxy(aryl substituted loweralkyl), carbamoyl(loweralkyl), alkoxycarbonyl(loweralkyl) or alkoxycarbonyl(aryl substituted loweralkyl) then the other of Y^2 and Y^3 is hydrogen or alkyl. Preferred for Y^2 and Y^3 are independently hydrogen, alkyl, cyano(loweralkyl), aryalkyl, heteroaralkyl, carboxy(loweralkyl), carboxy(aryl substituted loweralkyl) and carbamoyl(loweralkyl).

As used herein, the abbreviations for amino acids and protective groups are in accord with their common usage and the IUPAC-IUB Commission on Biochemical Nomenclature [see, (1972) Biochem. 11: 942-944]. Each naturally occurring L-amino acid is identified by the standard three letter code or the standard three letter code with or without the prefix "L-"; the prefix "D-" indicates that the stereoisomeric form of the amino acid is D. For example, as used herein, Ac is acetyl; Fmoc is 9-fluorenylmethoxycarbonyl; BOP is benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluorophosphate, DCC is dicyclohexylcarbodiimide; DDZ is dimethoxydimethylbenzyloxy; DMT is dimethoxytrityl; FMOC is fluorenylmethyloxycarbonyl; HBTU is 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium; hexafluorophosphate NV is nitroveratryl; NVOC is 6-nitroveratryloxycarbonyl and other photoremovable groups; TFA is trifluoroacetic acid; DMF for N,N-dimethylformamide; Boc is *tert*-butoxycarbonyl; ACN is acetonitrile,TFA for trifluoroacetic acid; HF for hydrogen fluoride; HFIP for hexafluoroisopropanol; HPLC for high performance liquid chromatography; FAB-MS for fast

atom bombardment mass spectrometry; DCM is dichloromethane, Bom is benzyloxymethyl; Pd/C is palladium catalyst on activated charcoal; DIC is diisopropylcarbodiimide; DCC is N,N'-dicyclohexylcarbodiimide; [For] is formyl; PyBop is benzotriazol-1-yl-oxy-trispyrrolidino-phosphonium hexafluorophosphate; POPOP is 1,4,-bis[5-phenyl(oxazolyl)benzene]; PPO is 2,5-diphenyloxazole; butyl-PBD is [2-(4'-tert-butylphenyl)-5-(4"-biphenyl)-1,3,4-oxadiazole]; PMP is (1-phenyl-3-mesityl-2-pyrazoline) DIEA is diisopropylethylamine; EDIA is ethyldiisopropylethylamine; NMP is N-methylpyrrolidone; NV is nitroveratryl PAL is pyridylalanine; HATU is O(7-azabenzotriaol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; TFA is trifluoracetic acid, THF is tetrahydrofuran; and EDT is 1,2-ethanedithiol.

As used herein, a tyrphostin analog refers to compounds that have a structure based on the pharmacophore core set forth in FIGURES 33 and 34 and elsewhere herein, particular compounds of formula (I). In addition, for purposes herein, preferred among these compounds are those that exhibit pharmacological activity based on tyrosine kinase inhibition that is at least as potent an anti-cancer agent as the compound known to those of skill in the art as tyrphostin AG490. Potency may be measured by any assay known to those of skill in this art or by the assay provided herein. Among the more preferred analogs are those that are less cytotoxic to normal cells than AG490 or that are less toxic (at least about 20%) to non-cancerous cells (cells that have lost contact inhibition) than to cancer cells. In particular, as used herein, analogs of typrohstin AG490 provided herein refer to compounds that have formula (I) the are produced by combining BB1, BB2 and optionally BB3 according the reaction scheme generally set forth in FIGURE 35.

II. Combinations of matrices with memory and tyrphostin-based compounds, screening methods, pharmaceutical compositions

Protein tyrosine kinases (PTKs) regulate cell proliferation, cell differentiation, and signaling processes in the cells of the immune system. Uncontrolled signaling from receptor tyrosine kinases and intracellular

tyrosine kinases can lead to inflammatory responses and to diseases, such as cancer, atherosclerosis, and psoriasis. Inhibitors that block the activity of tyrosine kinases and the signaling pathway they activate may provide a useful basis for drug development. Tyrphostins are a group of compounds that inhibit the PTKs. The tyrphostin designated AG490 selectively blocks leukaemic cell growth in vitro and in vivo by inducing programmed cell death, with no deleterious effect on normal haematopoiesis. To identify compounds with improved tyrphostin activity and lower cytoxicity, a procedure for generating an AG490 analog library is provided herein. The synthesis (Scheme I and II, FIGURES 35 and 36, respectivel) sequentially includes the steps of: a step of hooking a linker onto a suitably derivitized matrix material, preferably a matrix in combination with a memory; a capping step the extra amine on the matrix, a de-Fmoc step followed by a reductive alkylation, a secondary amine cyanoacetylation, an Aldol condensation, and an optional acetylation or aklyation; and a final cleavage with TFA in dioxane or benzene.

In an exemplified embodiments, three sets of building blocks for a 432 (18x8x3) member of library are provided. The building blocks include 18 aldehydes for reductive alkylation, 8 hydroxy aldehydes for the Aldol condensation and 3 building blocks (none, acetic chloride, benzoic chloride) for esterificarion. Two libraries (18x8x3=432) libraries have been synthesized. Building blocks for a 10K and more member library are also provided.

A. Tyrosine kinase inhibitors as anti-proliferative agents

One class of tyrosine kinase inhibitors that have use as with potential use as anti-proliferative agents. Among these agents are those that share a phenolic styrene pharmacophore. Of these tyrphostins are of interest herein. These compounds typically are characterized as incorporating the benzylidene moiety of erbstatin [see, e.g., Yaish et al. (1988) Science 242:933; Gazit et al. (1989) J. Med. Chem. 32:2344; and Gazit et al. (1991) J. Med. Chem. 34:1896]. Compounds that exhibit activity of

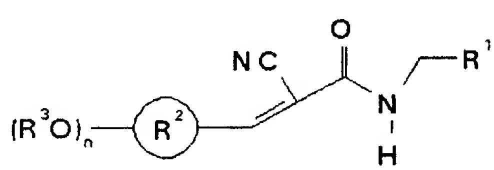
tyrphostin should have use in the treatement of hyperproliferative disorders, including certain cancers, psoriasis and inflammatory disorders and other such disorders enumerated herein and known to those of skill in the art. Tyrphostin AG490 analogs should be particularly promising candidates for treatment of leukemias and carcincomas with elevated EGF receptor expression.

Accordingly, libraries of compounds and methods for preparation of compounds based upon this pharmacophore structure are provided herein. In particular, solid phase methods for synthesis of such compounds in combination with matrices with memories are provided.

B. Combinations of tyrphositin-based compounds and matrices with memories and libraries containing such compounds

1. Compounds

Of particular interest herein are combinations of matrices with memory in which the associated molecules are compounds of Formula (I):



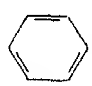
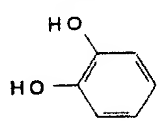
in which

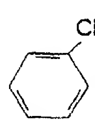
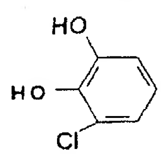
R^1 is selected from the alkyl, which may be straight or branched or cyclized containing one ring or two fused rings, or mixtures thereof, containing preferably 1 to 15 carbons, more preferably 1 to 6, and is more preferably aryl or heteroaryl, containing preferably from 5 to 7 members in the ring, and is more preferably phenyl, thienyl, furyl, pyrrolyl, phenyl, pyridinyl, morpholino, or pyrimidinyl; R^1 is unsubstituted or is substituted with one or more substituents selected from alkyl, cyano, halide, alkoxy, alkoxyalkyl, alkyl, thioxy, alkylthioxy, haloalky, OH, and amino;

R^2 is selected from the alkyl, which may be straight or branched or cyclized containing one ring or two fused rings, or mixtures thereof,

containing preferably 1 to 15 carbons, more preferably 1 to 6, and is more preferably aryl or heteroaryl, containing preferably from 5 to 7 members in the ring, and is more preferably phenyl, thienyl, furyl, pyrrolyl, phenyl, pyridinyl, morpholino, or pyrimidinyl; R^2 is unsubstituted or is substituted with one or more substituents selected from $(R^4)_p$, in which each R^4 is independently selected from alkyl, cyano, halide, alkoxy, alkoxyalkyl, alkyl, thioxy, alkylthioxy, haloalky, OH, and amino, where R^4 is preferably OH or halide or lower alkyl and n is preferably 0 to 2; and

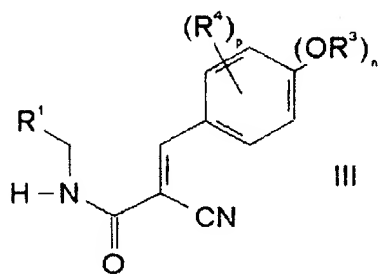
R^3 is from H, alkyl, acetyl, $CH_3(CH_2)_qC(O)$ where q is 0 to 3, aryl-$(CH_2)_qC(O)$ or heteroaryl-$(CH_2)_qC(O)$, which are unsubstituted or substituted with one or more substituents, such as R^4, which is preferably halide, lower alkyl, lower alkoxy, nitro, and preferably contain 5 to 7 members in the ring and is of formula aryl-C(O) or heteraryl-C(O). R^3 is more preferably acetyl, or includes an aryl or heteroaryl containing 5 or 6 members in the ring and one heteroatom, selected from among S, O, N, or aryl, containing 5 to 7 members in the ring or aryl, in which the aryl group is preferably a 5 or 6-membered ring, preferably phenyl and the heteroaryl is preferably selected from thienyl, furyl, pyrrolyl, phenyl, pyridinyl, morpholino, or pyrimidinyl, and more preferably is acetyl, and benzoyl. The compounds are selected with the proviso that when R^3 is H, then R^1 and R^2 are not:

 and  , respectively, or

 and  , respectively.

The excluded compounds are preferably not included in any library prepared according to the methods herein. Although if included in the library, particularly a library linked to matrices with memories, they will be readily identifiable, and, if desired can serve as controls in assays

Preferred among those compounds are those in which R^2 is phenyl and the resulting compounds have formula (III):



III

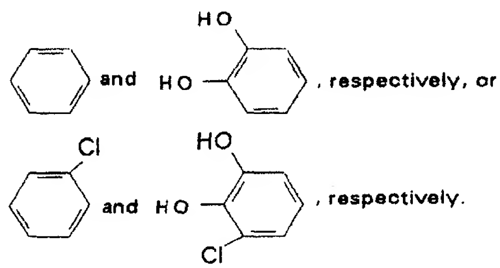
in which:

n is 0 to 3, preferably 1 or 2 and p is 0 to 3, preferably 1 or 2, as long as n + p is not greater than 5;

each R^4 is independently selected from among alkyl, cyano, halide, alkoxy, alkoxyalkyl, alkyl, thioxy, alkylthioxy, haloalky, hydroxy, nitro and amino;

R^3 is from H, alkyl, acetyl, $CH_3(CH_2)_qC(O)$ where q is 0 to 3, aryl-$(CH_2)_qC(O)$ or heteroaryl-$(CH_2)_qC(O)$, which are unsubstituted or substituted with one or more substituents, such as R^4, which is preferably halide, lower alkyl, lower alkoxy, nitro, and preferably contain 5 to 7 members in the ring and is of formula aryl-C(O) or heteraryl-C(O). R^3 is more preferably acetyl, or includes an aryl or heteroaryl containing 5 or 6 members in the ring and one heteroatom, selected from among S, O, N, or aryl, containing 5 to 7 members in the ring or aryl, in which the aryl group is preferably a 5 or 6-membered ring, preferably phenyl and the heteroaryl is preferably selected from thienyl, furyl, pyrrolyl, phenyl, pyridinyl, morpholino, or pyrimidinyl. R^3

is more preferably is acetyl and benzoyl. In all instances, the resulting compound is not the tyrphostin AG490, i.e. if BB3 is nothing (i.e. R^3 is H), then R^1 and R^2 cannot be:



Compounds of formula (III) where R^2 is a 5 or 6-membered heteroaryl group instead of a phenyl group are also among those preferred herein. Additional diversity is achieved by altering the position of OR^3.

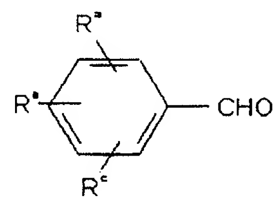
Preferred selections for R^1, R^2 and R^3 can be determined from BB1, BB2, and BB3, for which preferred selections are set forth in FIGURES 35-39. BB1 is R^1CHO; BB2 is a substituted phenol aldehyde; and BB3 is R^3-X, in which X is halide, preferably Cl or other suitable leaving group. BB1, BB2 and BB3 are commercially available compounds (i.e. from Aldrich and elsewhere) or may be readily synthesized by those of skill in this art.

BB1 is preferably an unsubstituted or substituted aromatic or heterocyclic aldehyde containing one or two fused rings. Preferred substituents include halide, nitro, alkyl, phenyl, alkoxy, hydroxy, phenoxy, or alkylenedioxy, alkylenethioxyoxy or alkylenedithioxy (i.e. $-O-(CH_2)_n-O-$, $-S-(CH_2)_n-O-$, $-S-(CH_2)_n-S-$.

BB3 is preferably nothing (where the resulting compound has an hydroxy group rather than R^3; see, Formula (I)) or is an acylating or

alkylating reagent that has the formula R^3-X, and includes 5 to 7 membered aryl or heteroaryl groups that are unsubstituted or substituted at one or more positions with halide, nitro, lower alkyl, hydroxy, alkoxy, or phenyl or other aryl group substituent.

Preferably, BB2 is an aryl or heterary group containing one ring or two fused rings, and is preferably a substituted phenol aldehyde that is substituted with one or more substituents each independently selected from H, OH, lower alkyl, lower alkoxy, alkoxyalkyl, and halo. More preferably, BB2 has the formula (IV):



,

where each of R^a, R^b and R^c are selected from among H, OH, alkyloxy, preferably lower alkyloxy, more preferably methoxy, halo, preferably bromo, nitro, with the proviso that at least one of R^a, R^b, and R^c is OH.

Preferred selections for each of BB1, BB2, and BB3 are set forth in FIGURES 35-39. R in Figure 39B can be any group and is preferably any of the substituents set forth for BB1, BB2, or BB3, and more preferably is an aryl group substituent as defined above, or any of the substituents set forth for R^a, R^b and R^c.

2. Libraries

Also provided are libraries containing combinations of matrices with memory and the compounds of Formula (I). The combinations provided herein thus have a multiplicity of applications, including isolation and purification of target macromolecules, capture and detection of macromolecules for analytical purposes, high throughput screening, selective removal of contaminants, drug delivery, chemical modification, information collection and management and other uses. These combinations

are particularly advantageous for use in multianalyte analyses, assays in which a electromagnetic signal is generated by the reactants or products in the assay, for use in homogeneous assays, and for use in multiplexed protocols.

The libraries are used for identifying compounds that have the ability to modulate, particularly inhibit, PTKs. The particular PTK can be chosen by using an appropriate screening protocol. When using the assay provided herein, the desired PTK will be used as the enzyme. The target receptor or substrate can be used as the substrate in the assay. Thus, the libraries while being designed around the AG490 pharmacophore, can contain compounds with a variety of activities and selectivities. Libraries synthesized using the sets of building blocks designated BB1, BB2 and BB3 or combinations and subsets of these substituents are provided. Libraries prepared from the building blocks in FIGURES 37 and 38 have been prepared and are provided. Compounds from the libraries are also provided.

Methods for tagging constituent members of combinatorial libraries containing compounds of Formula (I) are provided. These methods involve identifiably tagging the constituent members of the library by contacting the molecules or bringing such molecules into proximity with a matrix with memory and programming the memory with retrievable information from which the identity, synthesis history, batch number or other identifying information can be retrieved. In the preferred methods provided herein for tagging constituent members of libraries containing compounds of Formula (I), the memories are encoded during synthesis. of each compound. In one embodiment, the contacting is effected by bringing and maintaining the synthetic intermediate and/or end-product compound, linked to a matrix support, into proximity to the recording device by enclosing the device and matrix material in a microvessel. The memory associated with each distinct R group substituent is addressed, and the identity of the incorporated R group molecule is encoded in the memory at [before, during or after] each step in the synthesis. At the end of the synthesis, the memory contains a

retrievable record of all of the R group substituents of the resulting compound, which can then be used, either linked to the support, or preferably following cleavage from the support in screeninng assays and in assays to evaluate the properties of the compound or other such application.

Methods for synthesis and tagging and screening of libraries of matrices with memories and linked tyrphostin analogs are provided herein. In specific embodiments described herein for tagging constituent libraries of a desired number of members containing combinations of matrices with memory and the compounds of Formula (I), the starting compound is loaded onto suitably derivatized microreactors, which are preferably labeled with an electromagnetic tag or optical tag as described below. Synthesis proceeds according to the protocol outlined in FIGURE 35. The microvessels are split into pools corresponding to the number of unique members of BB1. Each pool is encoded with a unique RF or other code (or information that is associated with the tag on each memory with matrix stored in a remote memory) followed by coupling with the corresponding BB1, from which R^1 in formula (I) is generated, group constituent. The microvessels are combined and re-split into new pools equal to the number of BB2 groups (R^2) R groups to be added. The new pools are encoded (or information that is associated with the tag on each memory with matrix stored in a remote memory) according to the corresponding R^2 group or constitutent BB2 to be added. The microvessels are again combined and re-split for addition of R^3 (BB3). Exemplary synthetic scheme are set forth in FIGURES 35 and 36 and described in the EXAMPLES.

3. Practice of the method of preparation of a tyrphostin-based compound library using matrices with memories

a. Preparation of microreactors

In the exemplified embodiments MICROTUBE® microreactors are used. It is understood that any of the matrix with memory formats may be substituted therefor.

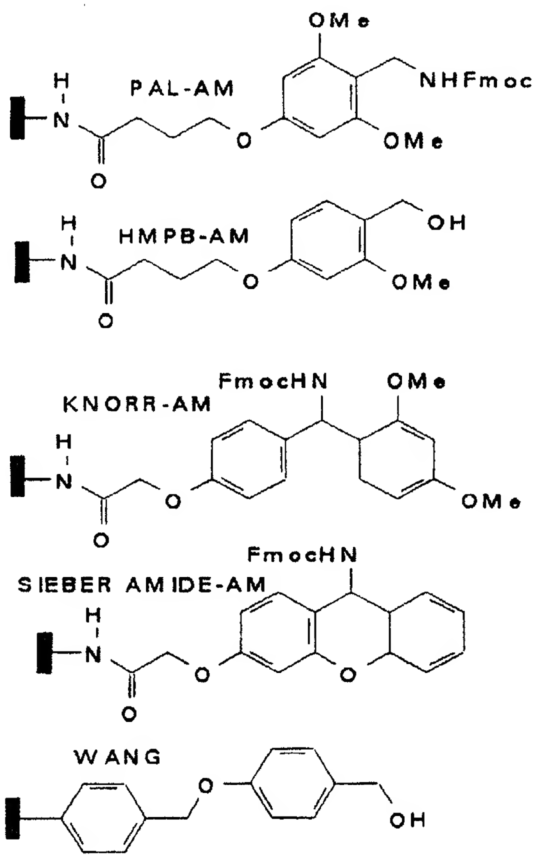
(1) Microreactors

The MICROTUBE® microreactors are prepared as as described below, and radiation grafted with polystyrene and chloromethylated and a suitable linker is attached. The solid phase matrix material is preferably polypropylene or PFTE, which has been surface-modified with polystyrene and suitably derivatized.

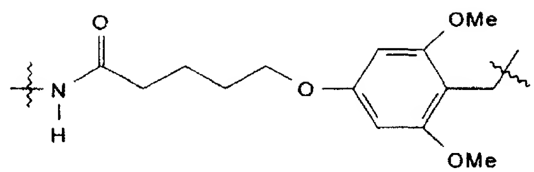
The memory device is preferably an electromagnetic tag contained within the matrix material. The device is introduced inside the matrix material or the material is wrapped around the device to yield matrix with memory "tubes" [e.g., MICROTUBES™, see, FIGURE 21]. Thus, polypropylene tubes or TEFLON are grafted and then formed into tubes or other suitable shape and the memory device inserted inside. The memory devices contained in these tubes are, for example, the IDT101 [IDTAG™ Ltd. Bracknell, Berks RG12 3XQ, UK] device described herein above, although the memory device that includes the modified form of the IPTT-100 described in herein may also be used 'or any other suitable tag is used.

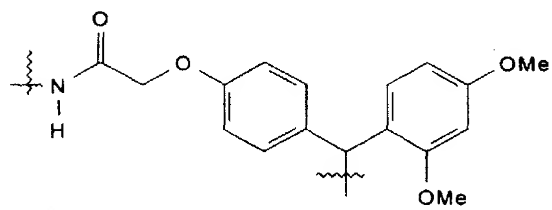
(2) Linkers

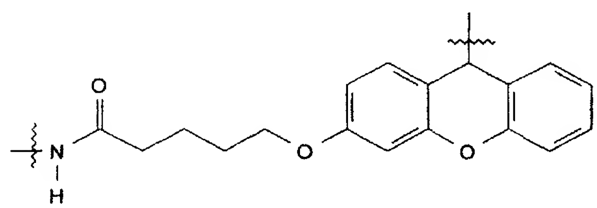
A linker functionality is attached to the matrix to serve as a means of linking the building blocks to the solid phase. A suitable linker is attached to the surface of the MICROTUBE microreacter. Such linkers, which are well known to those of skill in this art, include, but are not limited to, the acid-cleavable Fmoc-protected carboxamide Knorr linker (see, e.g., (Bernatowicz et al. (1989) Tetr. Lett. 30:4645-4648, PAL linker (Millipore or Biosearch; 5-(4-Fmoc-aminomethyl-3,5-dimethoxyphenoxy)valeric acid; Fmoc is 9-fluorenylmethyloxycarbonyl; Milligen); Seiber amide linker, HMPB (4(4-hydroxymethyl-3-methoxyphenoxy)butyric acid; see e.g., U.S. Patent No. 5,527,881); Rink linker (4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxy); and Wang linker (see, e.g., Wang (1976) J. Org. Chem. 41:32-58):



Acid-cleavable amine linkers are preferred in these methods and are particularly selected from the following:







Choromethylated linkers on the tubes can bere converted to Wang linkers as follows. In a typical experiment, a mixture of about 50 chloromethylated tubes (23 μmol/tube), 3-fold 4-hydroxybenzyl alcohol and 2.95-fold NaOMe in N,N-dimethylacetamid (75 ml) was heated at 50° C for 16 hr. After washing with dioxzane (2 times), dioxane/H_2O (1:1, 3 times), tetryhydrofuran (THF) and MeOH (1 time each) and THF (3 times), the resulting "Wang" MICROTUBE® microreactors were dried under vacuum.

b. Libraries

Tyrphostin-based compound libraries containing, e.g., 1,200,000 members or any desired number, are prepared using solid phase synthesis

methods that incorporate matrices with memories. In preferred methods of preparing the libraries, the matrices are the solid phase used in the synthesis of the member compounds. Building blocks, which are component parts of each compound, including various R groups, are added to the matrix in a step-wise fashion. Thus, synthesis begins with the matrix with memory combination and proceeds by sequential addition of building blocks, which is accompanied by an encoding of the memory at each addition of a R group. In this manner, each end-product compound of the synthesis will be readily indentifiable by its unique code corresponding to the R groups contained in the compound.

c. General synthetic scheme

Solid phase methods for synthesis of the compounds of Formula (I) are provided herein. Preferred methods utilize a polystyrene resin derivatized with an acid cleavable linker or other functionality as the solid phase in the synthesis.

The most preferred methods for preparing compounds of Formula (I) incorporate the above-described matrices with memories for identifiably tagging the compounds and/or intermediates in the synthesis thereof. As a result, distinct compounds of Formula (I) possessing specific R group substitutents are uniquely tagged during such synthesis. The matrices are used for the synthesis of each compound, the memory associated with each distinct R group substituent is addressed, and the identity of the incorporated R group molecule is encoded in the memory at [before, during, or after] each step in the synthesis. At the end of the synthesis, the memory contains a retrievable record of all of the R group substituents of the resulting compound, which can then be used, preferably following cleavage from the support and any further purification that may be required, in assays to evaluate the properties of the compound as anti-leukemic agents or other such application.

Practice of the methods is exemplified in the EXAMPLES. To prepare a tyrphostin-based compound library containing compounds of Formula (I), separate building blocks that include each of the desired three R groups are added to the matrix with memory tube. FIGURES 35 and 36, as well as 37-39, show preferred building blocks, referred to as BB1, BB2 and BB3, used to generate different R^1, R^2 and R^3 substituents, respectively, of each compound in a specific embodiment of a library of containing compounds of Formula (I). In the protocol depicted in FIGURE 35 using the building blocks in FIGURES 37-39, Knorr linkers were used. To generate a library with 1,200,000 members, a pool of 1,200,000 of the matrix with memory tubes (or other such device, as desired) would be prepared.

(1) Addition of BB1 - Synthesis of the linked amine compound

The first set of building blocks, **BB1**, are aldehydes with the desired R group for position R^1 of the final compound. The aldehyde moiety provides the means by which the accompanying R group is attached to the matrix through reaction with the amine of the acid-cleavable linker on the matrix. Specifically, to generate a library with 1,200,000 members, the pool of 1,200,000 matrix with memory tubes is split into 200 groups of 6000 members each and each pool is reacted with one of 200 **BB1** aldehydes by nucleophilic addition of an amine to form an imine as follows. The matrix tubes (microreactors) carrying an acid-cleavable linker are reacted with **BB1** in trimethylorthoformate at room temperature to give imines, which are reduced with $NaCNBH_3$ to yield the desired amines. FIGURE 36, which shows the synthesis a specific embodiment of the tyrphostin-based library, illustrates this step in generating **2** from **1**.

Prior, during or after addition of **BB1** to the matrix, the memory is encoded with a unique code, e.g., a radiofrequency code, corresponding to the individual **BB1** moiety.

(2) **Acylation of the amine compound**

Reaction of the amine formed in the previous step with cyanoacetic acid proceeds through a standard nucleophilic substitution pathway to give the acylated amine compound [see also Gazit et al. (1991) J. Med. Chem. 34:1896]. In the reaction scheme for the specific embodiment given in FIG. 36, this step is illustrated in the acylation of amine **2** to yield the cyanoacetoamide **3**.

(3) **Addition of BB2 - Reaction of the acylated amine with BB2**

Each of the 200 pools of 6000 members is combined and then split into 15 pools of 80,000 members each and the cyanoacetoamides of each pool are condensed with one of 15 **BB2** aldehydes in piperidine to yield α,β-unsaturated amides. The reaction proceeds through a Knoevenagel condensation [see also Gazit et al. (1991) J. Med. Chem. 34:1896] to yield the α-cyano ketone. In the reaction scheme for the scheme in Figure 35, this step is illustrated in the generation of **3** to **4**.

Prior, during or after addition of **BB2** to the pools, the memory of each matrix is encoded with a unique code, e.g., a radiofrequency code, corresponding to the individual **BB2** moiety being added.

(4) **Addition of BB3 - Acylation of the α-cyano ketone**

The above pools are then combined and split into 400 pools of 3000 members each and the phenolic group of each pool is reacted with one of 400 **BB3** alkyl or acyl halides to yield the corresponding ether or ester. In the reaction scheme for the specific embodiment given in FIG. 54, this step is illustrated by the generation of **5** from **4**. As noted, above, among the selections for BB3 is nothing. In which case the resulting tyrphostin analog will have a hydroxy group.

(5) **Isolation of compounds and Analytical data**

The matrix with memory tubes are then de-coded by reading the recorded codes (e.g., as can be done using a specifically designed radio

frequency memory retrieving device [such as the Bio Medic Data Systems Inc. DAS-5001 CONSOLE™ System, see, also U.S. Patent No. 5,252,962 and U.S. Patent No. 5,262,772]), distributed into 1,200,000 individual glass vials (or a subset thereof for pool screening) labeled with the RF or other codes, and treated with 2,2,2-trifluoroethanol and dichloromethane to cleave the final product from the matrix. Analytical data (electrospray MS, HPLC purity, and % yield) for the members of the library are determined. The compounds may be evaluated for biological activity according to assays as described in the EXAMPLES.

d. Exemplary embodiment

A particular embodiment of a tyrphositin library is set forth in detail in the EXAMPLES. To prepare the library, a set of 432 tube-shaped microreactors was prepared by : (a) radiation grafting polystyrene (ca. 350 umoles) on to 1" -long polyproplene tubes, (b) chemically functionalizing the polystyrene with aminomethyl groups to afford ca. 55 umoles of amine per reactor, (c) inserting one reusable radiofrequency ('Rf') ID tag into each tube, and (d) heat-sealing the tube ends. The chemical conversion of all 432 tubes was carried out simultaneously using standard procedures, affording reactors with ca. 50 umoles of available amine per tube.

A matrix notation is used describe sets of reagents serving as diversity inputs [e.g., 3(1-18)], an individual reagent from this set [e.g., 3(6)], product libraries [e.g., 12(1-18,1-8,1-3)], and an individual product [e.g.,(12,1,3)]. Each diversity-introducing reaction step is represented in order in the matrix descriptor. The adjective 'chemset' refers to a matrix of compounds, to include all the described members of a given compound matrix unless otherwise specified [e.g., chemset 6 is equivalent to 6(1-18,1-8,1-3)].

The first step (with reference to FIGURES 35, 36 and 37) is the reductive amination of the 1 to 2, using commercially available benzaldehydes (BB1). This set of conversions was accomplished in 18 reaction vessels, each containing 8x3=24 reactors. Thus, at the end of this

synthetic step, the product is 8x3 = 24 copies of each member of the chemset, which contains 18 members.

The conversion of 2 to 3 is not a diversity-introducing step, and so the reaction could be accomplished in one vessel containing all 432 microreactors. The product is thus 8x3 = 24 copies of each member of the next set 3, which contains 18 members. The conversion of 3 to 5 is the second diversity-introducing step, and was accomplished in 8 vessels containing 18x3 = 54 reactors each. In order to make every member of the next chemset 5, it is essential that three copies of each member of the set accurately isted deposited into each of the 8 vessels used to accomplish the conversion. Because the previous reaction step resulted in the loss of knowledge regarding which reactors bear which product, reading the RF ID tag is required to accomplish this process. A database assigning each reactor to a member of the final matrix of compounds provides the necessary encoding strategy. Once each reactor is identified using its ID tag, it can be physically placed into the correct vessel for the next reaction step. This process is designated herein as "directed sorting"; it is required in order to each member of a set of products once and only once. The product is thus 3 copies of each member of the next set, which contains 18x8 = 162 members. The conversion of chemset 5(1-18,1-8) to 6(1-18,1-8,1-3) is the third and last diversity-introducing step, and was accomplished in 3 vessels containing 18x8 = 162 reactors each.

The details of the reactions and this process are set forth in the EXAMPLES.

C. **Screening Assays and selection of tyrphostin analogs**

Any suitable assay known to those of skill in the art for screening for compounds that inhibit PTKs, preferably in conjunction with cytoxicity assays, may be used. The universal PTK assay provided herein and described in the EXAMPLES is preferred herein. Preferably this PTK assay is used with cytotoxicity assays to select activity PTK inhibitors that are

selectively cytoxtoxic (i.e., at least about 20% more cytotoxic to target cells or tissues than non-targeted cells or tissues).

Protein Tyrosine Kinase (PTK) Assay

Protein phosphorylation is a posttranslation modification of certain residues on proteins and plays an important role in signal transduction. When a particular protein is phosphorylated, a chain of events occur which result in changes in cell behavior or growth. Protein kinases are a class of enzymes that can transfer a phosphate from ATP to the hydroxyl on serine, threonine or tyrosine residues. Protein kinase activity is associated with stimulation of many growth factor receptors, proto-oncogene products, cancer, leukemia and disorders of the immune system. A subset of protein kinases are those thatphosphorylate specific tyrosine residues. The importance of PTKs in signal transductions and the association of aberrant PTKs expression with proliferative disorders makes agents that modulate the activity of PTKs attractive therapeutic targets and pharmaceutical probes. Thus, it is of considerable interest to develop set up an assay system that is specific, highly accurate and robotic friendly for inhibition of PTK activity. Such an assay system is provided herein.

In all phosphorylation reactions, the three required components are: tyrosine kinase, a substrate containing tyrosine, and ATP. In the exemplified embodiment, human p60$^{c\text{-}src}$, which is commercially available as a recombinant protein purified from baculovirus is used. The p60 c-src enzyme is related to the viral oncogene pp60$^{v\text{-}src}$ and has been extensively characterized. It will phosphorylate generic polymeric substrates (e.g. poly(gly,tyr) 4:1) and peptides specific for this enzyme. Typically, ATP containing a radiolabelled tracer (e.g. [Y-33P] ATP) is used for quantifying the phosphorylation reaction. The kinase transfers the radioactive phosphate from ATP to the tyrosine, which can be measured in a scintillation counter.

Two assays (1) a solution-based assay (Millipore Multiscreen plates) and (2) a scintillation proximity based assay (Wallac ScintiStrips plate) for

measuring tyrosine kinase activity a in a 96-well microplate format are provided. In the first assay, the reaction is initiated by adding all three components (p60$^{c\text{-}src}$, ATP, and poly[glu,tyr] 4:1]) to the wells of a Millipore Multiscreen plate. The Multiscreen plate contains a 0.22 μm Durapore membrane on the bottom of each well. After a four hour incubation, the reaction is stopped by the addition of an acid. This precipitates the phosphorylated polymer and enzyme. The plate is placed on a Multiscreen manifold and a vacuum is applied. The precipitate is captured by the membrane while unreacted ATP is removed. After adding scintillation cocktail to each well, the plate is counted.

In the second preferred assay, which is adaptable for robotics and is ideal for high throughput screening strategies, phosphorylation is performed on substrates bound to a solid support. ScintiStrips (Wallac) or other suitable support material, preferably in a 96 well or higher density format, that contains a scintillant embedded in the matrix material, such as polystyrene, is used as the support. The poly[glu,tyr] 4:1 substrate may be used to coat standard ScintiStrips directly, or it may be biotinylated and applied to ScintiStrips precoated with streptavidin (either covalently or noncovalently). The ATP and p60$^{c\text{-}src}$ are added to phosphorylate the substrate. After a four hour incubation, the supernatant (containing unreated ATP and enzyme) is removed by aspiration. The ScintiStrips can then be counted without having to add scintillation cocktail.

ScintiStrips or other such supports are preferred over the solution phase assay because they are easier to handle and work up, and the procedure can be adapted robotics. Three types coated ScintiStrips have been successfully used for the phosphorylation of poly[glu,tyr] 4:1, and streptavidin coated ScintiStrips were selected for further optimization studies. Comparison of substrate concentration and coating time showed that an overnight incubation of 0.1 mg/mL solution of poly[glu,tyr] 4:1 conjugated with biotin-NHS was optimal for coating ScintiStrips. The reaction was initiated when 3 μM ATP (containing -40,000-60,000 counts)

and 2 units of $p60^{c\text{-}src}$ enzyme was added to each well. The plate was incubated at room temperature and reaction times were compared. A four hour reaction time was sufficient to transfer >60% of the counts to the bound substrate. Finally comparison of wash buffers showed that low background counts were obtained when the wells were washed with solutions containing strong acid, high salt, or detergent. A wash buffer containing 2 M NaCl and 0.5% $Na_4P_2O_7$ was selected.

This solid phase assay using streptavidin coated ScintiStrips and biotinylated substrate has many advantages over other methods used to measure kinase activity. First, streptavidin ScintiStrips are commercially available in a 96 well microplate format. Second, the scintillant is an integral part of the ScintiStrips; thus, no scintillation cocktail is required. Third, since the scintillant is embedded in the plate, ScintiStrips or other such plates, are easy to handle and wash, even under harsh conditions. Fourth, only the biotinylated substrate is captured by the streptavidin and counted when phosphorylated with [Y-33P] ATP. Phosphorylated tyrosine residues on nonbiotinylated substrates will not be counted. This is an important consideration when crude extracts containing impure tyrosine kinases are used, since nonbiotinylated material will not bind. Therefore, streptavidin ScintiStrips or the equivalent support can be used as part of a general assay system for measuring the activity of any pure or impure tyrosine kinase using any biotinylated substrate of interest.

This assay can be adapted for high throughput screening of tyrosine kinase inhibitors. The same general procedure may be used, except that library compounds are also added to the wells. Fewer counts are measured in wells containing compounds that inhibit tyrosine kinase activity.

The assay may be modified to identify compounds that selectively inhibit other PTKs, such as by substituing another PTK for $p60^{c\text{-}src}$, with another Src family member, a Csk family member, a Syk family member, a Btk family member, an Abl family member, an Fps family member, or a Jak

family member. Preferred PTKs are non-receptor PTKs, which are each differentiallly expressed in different tissues.

D. Pharmaceutical compositions

Provided herein are pharmaceutical compositions containing the compounds of Formula (I). A compound of formula (I) selected for a desired indication is formulated in a suitable vehicle for a particular indication.

1. Compounds of Formula (I)

Compounds of Formula (I) possess antitumor and/or antiproliferative and/or antiinflammatory properties or other properties possessed by PTK inhibitors. The compounds can have inhibitory effects on the survival of cancer cells and act as antineoplastic agents. Thus, further provided herein are methods of treating neoplastic diseases, including but not limited to leukemia and carcinomas. The methods use compounds of Formula (I) as chemotherapeutic agents. In particular, methods of treating patients suffering from leukemias, such as chronic myelogenous leukemia and acute lymphoblastic leukemia, are effected by administering an effective amount of the pharmaceutical compositions provided herein.

2. Pharmaceutical compositions containing compounds of Formula (I)

Pharmaceutical compositions containing compounds of Formula (I) are also provided. Effective concentrations of one or more of the compounds of Formula (I) or pharmaceutically acceptable salts, esters or other derivatives thereof are mixed with a suitable pharmaceutical carrier or vehicle. In instances in which the compounds exhibit insufficient solubility, methods for solubilizing compounds may be used. Such methods are known to those of skill in this art, and include, but are not limited to, using cosolvents, such as dimethylsulfoxide (DMSO), using surfactants, such as tween, or dissolution in aqueous sodium bicarbonate. Derivatives of the compounds, such as salts of the compounds or prodrugs of the compounds may also be used in formulating effective pharmaceutical compositions.

The concentrations of the compounds are effective for delivery of an amount, upon administration, that ameliorates the symptoms of the neoplastic disease. Typically, the compositions are formulated for single dosage administration.

Upon mixing or addition of the compound(s), the resulting mixture may be a solution, suspension, emulsion or the like. The form of the resulting mixture depends upon a number of factors, including the intended mode of administration and the solubility of the compound in the selected carrier or vehicle. The effective concentration is sufficient for ameliorating the symptoms of the disease, disorder or condition treated and may be empirically determined.

Pharmaceutical carriers or vehicles suitable for administration of the compounds provided herein include any such carriers known to those skilled in the art to be suitable for the particular mode of administration. In addition, the compounds may be formulated as the sole pharmaceutically active ingredient in the composition or may be combined with other active ingredients.

The active compounds can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid, semi-liquid or solid form and are formulated in a manner suitable for each route of administration. Preferred modes of administration include oral and parenteral modes of administration.

The active compound is included in the pharmaceutically acceptable carrier in an amount sufficient to exert a therapeutically useful effect in the absence of undesirable side effects on the patient treated. The therapeutically effective concentration may be determined empirically by testing the compounds in known in vitro and in vivo systems and then extrapolated therefrom for dosages for humans.

The concentration of active compound in the drug composition will depend on absorption, inactivation and excretion rates of the active

compound, the dosage schedule, and amount administered as well as other factors known to those of skill in the art.

Typically a therapeutically effective dosage should produce a serum concentration of active ingredient of from about 0.1 ng/ml to about 50-100 μg/ml. The pharmaceutical compositions typically should provide a dosage of from about 0.01 mg to about 2000 mg of compound per kilogram of body weight per day. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at intervals of time. It is understood that the precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by extrapolation from in vivo or in vitro test data. It is to be noted that concentrations and dosage values may also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed compositions.

If oral administration is desired, the compound should be provided in a composition that protects it from the acidic environment of the stomach. For example, the composition can be formulated in an enteric coating that maintains its integrity in the stomach and releases the active compound in the intestine. The composition may also be formulated in combination with an antacid or other such ingredient.

Oral compositions will generally include an inert diluent or an edible carrier and may be compressed into tablets or enclosed in gelatin capsules. For the purpose of oral therapeutic administration, the active compound or compounds can be incorporated with excipients and used in the form of tablets, capsules or troches. Pharmaceutically compatible binding agents and adjuvant materials can be included as part of the composition.

The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder, such as microcrystalline cellulose, gum tragacanth and gelatin; an excipient such as starch and lactose, a disintegrating agent such as, but not limited to, alginic acid and corn starch; a lubricant such as, but not limited to, magnesium stearate; a glidant, such as, but not limited to, colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; and a flavoring agent such as peppermint, methyl salicylate, and fruit flavoring.

When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar and other enteric agents. The compounds can also be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

The active materials can also be mixed with other active materials which do not impair the desired action, or with materials that supplement the desired action, such as antacids, H2 blockers, and diuretics. For example, use of the compound for treating antineoplastic disease may involve co-therapy with other anticancer agents, including, but not limited to, cisplatin.

Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include any of the following components: a sterile diluent, such as water for injection, saline solution, fixed oil, polyethylene glycol, glycerine, propylene glycol or other synthetic solvent; antimicrobial agents, such as benzyl alcohol and methyl parabens; antioxidants, such as ascorbic acid and sodium bisulfite; chelating agents, such as ethylenediaminetetraacetic acid (EDTA); buffers, such as acetates, citrates and phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose. Parenteral preparations can be enclosed in

ampules, disposable syringes or multiple dose vials made of glass, plastic or other suitable material.

If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof. Liposomal suspensions, including tissue-targeted liposomes, may also be suitable as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art. For example, liposome formulations may be prepared as described in U.S. Patent No. 4,522,811.

The compound may be linked to a targeting agent, such as a growth factor or monoclonal antibody for specific interaction with a targeted tissue or cell type (see, e.g., U.S. Patent No. 5,587,459, which describes preparation of immunoconjugates that contain a PTK inhibitor linked to a ligand that targets a cell surface receptor).

The active compounds may be prepared with carriers that protect the compound against rapid elimination from the body, such as time release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, implants and microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as collagen, ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and others. Methods for preparation of such formulations are known to those skilled in the art.

The compounds may be formulated for local or topical application, such as for topical application to the skin and mucous membranes, such as in the eye, in the form of gels, creams, and lotions and for application to the eye or for intracisternal or intraspinal application. Such solutions, particularly those intended for ophthalmic use, may be formulated as 0.01% - 10% isotonic solutions, pH about 5-7, with appropriate salts. The compounds may be formulated as aeorsols for topical application, such as by inhalation (see, e.g., U.S. Patent Nos. 4,044,126, 4,414,209, and

4,364,923, which describe aerosols for delivery of a steroid useful for treatment inflammatory diseases, particularly asthma).

Finally, the compounds may be packaged as articles of manufacture containing packaging material, a compound provided herein, which is effective for ameliorating the symptoms of neoplastic disease within the packaging material, and a label that indicates that the compound or salt thereof is used for treating neoplastic disease.

III. Combinations of matrices with memories

The following sections generally describe matrices with memory combinations and uses thereof. The methods, components and processes may be used with the particular embodiments of tyrphostin libraries, compounds and methods provided herein.

A. Matrices

Matrices, which are generally insoluble materials used to immobilize ligands and other molecules, have application in many chemical syntheses and separations. Matrices are used in affinity chromatography, in the immobilization of biologically active materials, and during chemical syntheses of biomolecules, including proteins, amino acids and other organic molecules and polymers. The preparation of and use of matrices is well known to those of skill in this art; there are many such materials and preparations thereof known. For example, naturally-occurring matrix materials, such as agarose and cellulose, may be isolated from their respective sources, and processed according to known protocols, and synthetic materials may be prepared in accord with known protocols.

Matrices include any material that can act as a support matrix for attachment of the molecules or biological particles of interest and can be in contact with or proximity to or associated with, preferably encasing or coating, the data storage device with programmable memory. Any matrix composed of material that is compatible with and upon or in which chemical syntheses are performed, including biocompatible polymers, is suitable for use herein. The matrix material should be selected so that it does not

interfere with the chemistry or biological reaction of interest during the time which the molecule or particle is linked to, or in proximity therewith [see, e.g., U.S. Patent No. 4,006,403]. These matrices, thus include any material to which the data storage device with memory can be attached, placed in proximity thereof, impregnated, encased or otherwise connected, linked or physically contacted. Such materials are known to those of skill in this art, and include those that are used as a support matrix. These materials include, but are not limited to, inorganics, natural polymers, and synthetic polymers, including, but are not limited to: cellulose, cellulose derivatives, acrylic resins, glass that is derivatized to render it suitable for use a support, silica gels, polystyrene, gelatin, polyvinyl pyrrolidone, co-polymers of vinyl and acrylamide, polystyrene cross-linked with divinylbenzene or the like [see, Merrifield (1964) Biochemistry 3:1385-1390], polyacrylamides, latex gels, polystyrene, dextran, polyacrylamides, rubber, silicon, plastics, nitrocellulose, celluloses, natural sponges, and many others. It is understood that the matrix materials contemplated are those that are suitable for use a s support matrix for retaining molecules or biological particles during synthesese or reactions.

Among the preferred matrices are polymeric beads, such as the TENTAGEL® resins and derivatives thereof [sold by Rapp Polymere, Tubingen, Germany; see, U.S. Patent No. 4,908,405 and U.S. Patent No. 5,292,814; see, also Butz et al. (1994) Peptide Res. 7:20-23; Kleine et al. (1994) Immunobiol. 190:53-66; see, also Piskin et al. (1994), Chapter 18 "Nondegradable and Biodegradable Polymeric Particles" in Diagnostic Biosensor Polymers, ACS Symp.Series 556, Usmani et al. Eds, American Chemical Society, Washington, DC], which are designed for solid phase chemistry and for affinity separations and purifications. See, also Bayer et al. (1994) in Pept.: Chem., Struct. Biol., Proc. Am. Pept. Symp., 13th; Hodges, et al. eds., pp.156-158; Zhang et al. (1993) Pept. 1992, Proc. Eur. Pept. Symp., 22nd, Schneider, et al., eds. pp. 432-433; Ilg et al. (1994) Macromolecules, pp. 2778-83; Zeppezauer et al. (1993) Z. Naturforsch., B:

Chem. Sci. 48:1801-1806; Rapp et al. (1992) Pept. Chem. 1992, Proc. Jpn. Symp., 2nd, Yanaihara, ed., pp. 7-10; Nokihara et al. (1993) Shimadzu Hyoron 50:25-31; Wright et al. (1993) Tetrahedron Lett. 34:3373-3376; Bayer et al. (1992) Poly(Ethylene Glycol) Chem. Harris, ed., pp. 325-45; Rapp et al. (1990) Innovation Perspect. Solid Phase Synth. Collect. Pap., Int. Symp., 1st, Epton, ed., pp. 205-10; Rapp et al. (1992) Pept.: Chem. Biol., Proc. Am. Pept. Symp., 12th, Smith et al., eds., pp. 529-530; Rapp et al. (1989) Pept., Proc. Eur. Pept. Symp., 20th, Jung et al., ed.,pp. 199-201; Bayer et al. (1986) Chem. Pept. Proteins 3: 3-8; Bayer et al. (1983) Pept.: Struct. Funct., Proc. Am. Pept. Symp., 8th, Hruby et al. eds.,pp. 87-90 for descriptions of preparation of such beads and use thereof in synthetic chemistry. Matrices that are also contemplated for use herein include fluophore-containing or -impregnated matrices, such as microplates and beads [commercially available, for example, from Amersham, Arlington Heights, IL; plastic scintillation beads from NE (Nuclear Technology, Inc., San Carlos, CA), Packard, Meriden, CT]. It is understood that these commercially available materials will be modified by combining them with memories, such as by methods described herein.

The matrix may also be a relatively made from inert polymer, which can be grafted by ionizing radiation [see, e.g., Figure 21, which depicts a particular embodiment] to permit attachment of a coating of polystyrene or other such polymer that can be derivatized and used as a support. Radiation grafting of monomers allows a diversity of surface characteristics to be generated on plasmid supports [see, e.g., Maeji et al. (1994) Reactive Polymers 22:203-212; and Berg et al. (1989) J. Am. Chem. Soc. 111:8024-8026]. For example, radiolytic grafting of monomers, such as vinyl momomers, or mixtures of monomers, to polymers, such as polyethylene and polypropylene, produce composites that have a wide variety of surface characteristics. These methods have been used to graft polymers to insoluble supports for synthesis of peptides and other molecules, and are of particular interest herein. The recording devices, which are often coated

with a plastic or other insert material, can be treated with ionizing radiation so that selected monomers can be grafted to render the surface suitable for chemical syntheses.

Where the matrix particles are macroscopic in size, such as about at least 1 mm in at least one dimension, such bead or matrix particle or continuous matrix may contain one or more memories. Where the matrix particles are smaller, such as NE particles [PVT-based plastic scintillator microsphere], which are about 1 to 10 μm in diameter, more than one such particle will generally be associated with one memory. Also, the bead, plate, vessel, container or particle may include additional material, such as scintillant or a fluophore impregnated therein. In preferred embodiments, the solid phase chemistry and subsequent assaying may be performed on the same bead or matrix with memory combination. All procedures, including synthesis on the bead and assaying and analysis, can be automated.

The matrices are typically insoluble substrates that are solid, porous, deformable, or hard, and have any required structure and geometry, including, but not limited to: beads, pellets, disks, capillaries, hollow fibers, needles, solid fibers, random shapes, thin films and membranes. Typically, when the matrix is particulate, the particles are at least about 10-2000 μM, but may be smaller, particularly for use in embodiments in which more than one particle is in proximity to a memory. For purposes herein, the support material will typically encase or be in contact with the data storage device, and, thus, will desirably have at least one dimension on the order of 1 mm [1000 μM] or more, although smaller particles may be contacted with the data storage devices, particularly in embodiments in which more than one matrix particle is associated, linked or in proximity to one memory or matrix with memory, such as the microvessels [see, e.g., FIGS. 11-16]. Each memory will be in associated with, in contact with or proximity to at least one matrix particle, and may be in contact with more than one. As smaller semiconductor and electronic or optical devices become available, the

capacity of the memory can be increased and/or the size of the particles can be decreased. For example, presently, 0.5 micron semiconductor devices are available. Integrated circuits 0.25-micron in size have been described and are being developed using a technology called the Complementary Metal Oxide-Semiconductor process (see, e.g., Investor's Business Daily 5/30/95).

Also of interest herein, are devices that are prepared by inserting the recording device into a "tube" [see, e.g., Figure 21] or encasing them in an inert material [with respect to the media in which the device will be in contact]. This material is fabricated from a plastic or other inert material. Preferably prior to introducing [and preferably sealing] the recording device inside, the tube or encasing material is treated with ionizing radiation to render the surface suitable for grafting selected monomers, such as styrene [see, e.g., Maeji et al. (1994) Reactive Polymers 22:203-212; Ang et al. in , Chapter 10: Application of Radiation Grafting in Reagent Insolubilization, pp 223-247; and Berg et al. (1989) J. Am. Chem. Soc. 111:8024-8026].

Recording device(s) is(are) introduced inside the material or the material is wrapped around the device and the resulting matrix with memory "tubes" [MICROTUBES™, see, FIGURE 21] are used for chemical synthesis or linkage of selected molecules or biological particles. These "tubes" are preferably synthesized from an inert resin, such as a polypropylene resin [e.g., a Moplen resin, V29G PP resin from Montell, Newark DE, a distributor for Himont, Italy]. Any inert matrix that can then be functionalized or to which derivatizable monomers can be grafted is suitable. Preferably herein, polypropylene tubes are grafted and then formed into tubes or other suitable shape and the recording device inserted inside. These tubes [MICROTUBES™] with grafted monomers are then used as synthesis, and/or for assays or for multiplexed processes, including synthesis and assays or other multistep procedures.

Such tubes may also have snap on or screw tops so that the memory device or chip is removable. For example, they may be conical tubes like

Eppendorf tubes, with a snap on top, preferably a flat top. The tubes will be of a size to accommodate a memory device and thus may be as small as about 2 mm x 2 mm x 0.1 to hold the small 2 mm x 2 mm x 0.1 mm device described herein. They will be fabricated from polypropylene or other suitable material and radiation grafted, see above, and Examples, below, preferably prior to introduction of the memory device.

Solid tubular embodiments, such as the MICROTUBE® microreactors made of tubes [or other geometry] have been coated or grafted with suitable materials are used as a solid support for any other methods disclosed herein, including organic syntheses and assays. Fluorophores, scintillants and other such compounds may also be incorporated into the surface or linked thereto [see, EXAMPLES below]. These tubes include those that contain the memory encased either permanently or removably or that include an imprinted symbology.

Briefly, for radiation-induced graft copolymerization, for example, of styrene to polypropylene (PP), polyethylene (PE) and teflon (PTFE) tubes, the diameter of the tube can be any desired size, with 0.1 mm to 20 mm presently preferred and 2 mm to 5 mm more preferred. It has been found that dilution of styrene with methanol enhances the rate of grafting, thereby permitting use of PTFE tubes. Dilutions, which can be determined empirically for each material, from 5% to 70 % have been tested. PTFE and PE tubes have the highest styrene grafting at a 50 % dilution, and polypropylene tubes have the best performance when grafted at a 35 % dilution. To effect grafting the polymer tubes are irradiated under a Co^{60} source. The dose rate can be empirically determined. Rates of 0.01×10^6 to 1×10^6 rads (r)/h are typical and the most effective rate was 0.1×10^6 r/h. A total dose of $0.5\text{-}10 \times 10^6$ rads was typical and the most effective dose was $2.6\text{-}2.9 \times 10^6$ rads.

Functional groups are introduced by selection of the monomers, such as styrene, choloromethylstyrene, methylacrylate, 2-hydroxymethylacrylate and/or other vinyl monomers containing one or more functional groups. For

example (see, e.g., FIGURES 33) aminomethyl functional groups been introduced by first radiation grafting polystyrene onto the surface of tubes of the above-noted polymers tubes followed by functionalization using N-(hydroxymethyl)phthalimide with trifluoromethanesulfonic acid as a catalyst. The polystyrene grafted polymer tube is thoroughly washed before use to remove residual monomer, non-attached polystyrene and additives remaining from the radiation grafting. The amidoalkylation proceeds smoothly at room temperature in 50 % (v/v) trifluoroacetic acid-dichloromethane solvent for 24 hours. Loading can be controlled by changing the concentrations of reagent, catalyst and/or reaction time. Hydrazinolysis in refluxing ethanol gives the aminomethyl polystyrene grafted polymer tube. Adjustable loading range is on the order of 0.5 - 100 μmol per tube, depending the size of the tube and the polymer.

A carboxylic acid group was introduced by using acrylate acid or functionalization of polystyrene. The polystyrene grafted tube was functionalized using *n*-butylithium and N,N N',N'-tetramethylethylendiamine in hexane at 60° C, after which the polymer tube was bubbled with CO_2. The carboxylic acid loading was about 1-20 μmol per tube.

Also larger matrix particles, which advantageously provide ease of handling, may be used and may be in contact with or proximity to more than one memory (i.e., one particle may have a plurality of memories in proximity or linked to it; each memory may programmed with different data regarding the matrix particle, linked molecules, synthesis or assay protocol, etc.]. Thus, so-called macro-beads (Rapp Polymere, Tubingen, Germany), which have a diameter of 2 mm when swollen, or other matrices of such size, are also contemplated for use herein. Particles of such size can be readily manipulated and the memory can be readily impregnated in or on the bead. These beads (available from Rapp) are also advantageous because of their uniformity in size, which is useful when automating the processes for electronically tagging and assaying the beads.

The matrices may also include an inert strip, such as a polytetrafluoroethylene [TEFLON®] strip or other material to which the molecules or biological particles of interest do not adhere, to aid in handling the matrix, such as embodiments in which a matrix with memory and linked molecules or biological particle are introduced into an agar-containing plate for immunoassays or for antibiotic screening.

Selection of the matrices will be governed, at least in part, by their physical and chemical properties, such as solubility, functional groups, mechanical stability, surface area swelling propensity, hydrophobic or hydrophilic properties and intended use.

The data storage device with programmable memory may be coated with a material, such as a glass or a plastic, that can be further derivatized and used as the support or it may be encased, partially or completely, in the matrix material, such as during or prior to polymerization of the material. Such coating may be performed manually or may be automated. The coating can be effected manually or using instruments designed for coating such devices. Instruments for this purpose are available [see, e.g., the Series C3000 systems for dipping available from Specialty Coating Systems, Inc., Indianapolis, IN; and the Series CM 2000 systems for spray coating available from Integrated Technologies, Inc. Acushnet, MA].

The data storage device with memory may be physically inserted into the matrix material or particle. It also can be manufactured with a coating that is suitable for use as a matrix or that includes regions in the coating that are suitable for use as a matrix. If the matrix material is a porous membrane, it may be placed inside the membrane. It is understood that when the memory device is encased in the matrix or coated with protective material, such matrix or material must be transparent to the signal used to program the memory for writing or reading data. More than one matrix particle may be linked to each data storage device.

In some instances, the data storage device with memory is coated with a polymer, which is then treated to contain an appropriate reactive

moiety or in some cases the device may be obtained commercially already containing the reactive moiety, and may thereby serve as the matrix support upon which molecules or biological particles are linked. Materials containing reactive surface moieties such as amino silane linkages, hydroxyl linkages or carboxysilane linkages may be produced by well established surface chemistry techniques involving silanization reactions, or the like. Examples of these materials are those having surface silicon oxide moieties, covalently linked to gamma-aminopropylsilane, and other organic moieties; N-[3-(triethyoxysilyl)propyl]phthelamic acid; and bis-(2-hydroxyethyl)aminopropyltriethoxysilane. Exemplary of readily available materials containing amino group reactive functionalities, include, but are not limited to, para-aminophenyltriethyoxysilane. Also derivatized polystyrenes and other such polymers are well known and readily available to those of skill in this art [e.g., the TENTAGEL® Resins are available with a multitude of functional groups, and are sold by Rapp Polymere, Tubingen, Germany; see, U.S. Patent No. 4,908,405 and U.S. Patent No. 5,292,814; see, also Butz et al. (1994) Peptide Res. 7:20-23; Kleine et al. (1994) Immunobiol. 190:53-66].

The data storage device with memory, however, generally should not or cannot be exposed to the reaction solution, and, thus, must be coated with at least a thin layer of a glass or ceramic or other protective coating that does not interfere with the operation of the device. These operations include electrical conduction across the device and transmission of remotely transmitted electromagnetic radiation by which data are written and read. It is such coating that may also serve as a matrix upon which the molecules or biological particles may be linked.

The data storage devices with memory may be coated either directly or following coating with a ceramic, glass or other material, may then be coated with agarose, which is heated, the devices are dipped into the agarose, and then cooled to about room temperature. The resulting glass, silica, agarose or other coated memory device, may be used as the matrix supports for chemical syntheses and reactions.

Conventional integrated circuit manufacturing and packaging methods include methods and means for encapsulating integrated circuits to protect the devices from the environment and to facilitate connection to external devices. Also, there are numerous descriptions for the preparation of semiconductor devices and wires, particularly for use as sensors [see, e.g., U.S. Patent No. 4,933,285; see, also Cass, Ed. (1990) Biosensors A Practical Approach, IRL Press at Oxford University Press, Oxford; biosensors are chemosensors an can include a biological detection system, generally biologically active substances, such as enzymes, antibodies, lectins and hormone receptors, which are immobilized on the surface of the sensor electrode or in a thin layer on the sensor electrode; biosensors are sensors that detect biological species], which measure electrochemical solution parameters, such as pH. Despite differences in the components of biosensors and recording devices used herein, certain of the methods for coating electrodes and wires in the biosensor art may be adapted for use herein [see, e.g., U.S. Patent Nos. 5,342,772, 5, 389,534, 5,384,028, 5,296,122, 5,334,880, 5,311,039, 4,777,019, 5,143,854, 5,200,051, 5,212,050, 5,310,686, 5324,591; see, also Usmani et al., ed. (1994) Diagnostic Biosensor Polymers, ACS Symposium Series No. 556].

It is, however, emphasized that the combinations herein of matrix with memory are not sensors, which measure external parameters and can include electrodes that must be in contact with the solution such that molecules in solution directly contact the electrode, and which measure solution parameters. Data regarding the combination, particularly the linked or associated biological particle or matrix is written into the memory, and thus records information about itself. Sensors monitor what is going outside of the device. The combinations herein of matrices with memories can be enhanced by addition of sensor elements for the measurement of external conditions, information about the external conditions can be recorded into the combination's memory. Such enhancements are contemplated herein.

The combinations herein are matrix materials with recording devices that contain data storage units (memories) that include remotely programmable memories; the recording devices used in solution must be coated with a material that prevents contact between the recording device and the medium, such as the solution or air or gas [e.g., nitrogen or oxygen or CO_2]. The recording device used in proximity to the matrix is preferably a miniature device, typically less than 10-20 mm^3 [i.e. up to 10-20 mm in each dimension, or 10-20 mm in its largest dimension] in size, preferably smaller, such as 1 to 10 mm on a side, that includes at least one data storage unit that includes a remotely programmable and remotely readable, preferably non-volatile, memory. This device with remotely programmable memory is in proximity to, associated with or in contact with the matrix. In particular, the recording device includes a memory device, *preferably having memory means*, preferably non-volatile, for storing a plurality of data points and means for receiving a transmitted signal that is received by the device and for causing a data point corresponding to the data signal to be permanently stored within the memory means. If needed, the recording device further includes a shell [coating] that is non-reactive with and impervious to any processing steps or solutions in which the combination of matrix with recording device [matrix with memory] is placed, and that is transmissive of read or write signals transmitted to the memory. The device may also include at least one support matrix disposed on an outer surface of the shell for retaining molecules or biological particles. The shell and support matrix may be the same. In such instances, the shell must be treated or derivatized such that molecules, particularly amino acids and nucleic acids, can be linked, preferably either electrostatically or covalently, thereto. Thus, a transponder enclosed in plastic, must be further treated or coated to render it suitable for linkage of the molecule or biological particle.

The information is introduced into the memory by addressing the memory to record information regarding molecules or biological particles linked thereto. Except in the reaction detecting [verifying] embodiment, in which the memory can be encoded upon reaction of a linked molecule or biological particle, solution parameters are not recorded in the memory.

In certain embodiments herein, the matrices with memories herein, however may be combined with devices or components or biosensors or other such sensor devices and used in connection therewith to monitor solution or external parameters. For example, the combination may be electronically or otherwise linked to a biosensor and information obtained by the biosensor can be encoded in memory, or the combination can transmit information to the biosensor or, when used internally in an animal, to monitor the location of a biosensor or to transmit information from the biosensor. For example, transponder memory devices exemplified herein, include circuitry for measuring and recording solution temperature. These transponders can be modified to read and record pH, instead of or in addition to temperature. Thus, during synthesis or other processing steps of linked or proximate molecules or biological particles, RF or other EM radiation will be used to encode information in the memory and at the same time pH and/or temperature in the external solution can be measured and recorded in the memory.

1. Natural matrix support materials

Naturally-occurring supports include, but are not limited to agarose, other polysaccharides, collagen, celluloses and derivatives thereof, glass, silica, and alumina. Methods for isolation, modification and treatment to render them suitable for use as supports is well known to those of skill in this art [see, e.g., Hermanson et al. (1992) Immobilized Affinity Ligand Techniques, Academic Press, Inc., San Diego]. Gels, such as agarose, can be readily adapted for use herein. Natural polymers such as polypeptides, proteins and carbohydrates; metalloids, such as silicon and germanium, that have semiconductive properties, as long as they do not interfere with

operation of the data storage device may also be adapted for use herein. Also, metals such as platinum, gold, nickel, copper, zinc, tin, palladium, silver, again as long as the combination of the data storage device with memory, matrix support with molecule or biological particle does not interfere with operation of the device with memory, may be adapted for use herein. Other matrices of interest include oxides of the metal and metalloids such as Pt-PtO, Si-SiO, Au-AuO, TiO2, Cu-CuO, and the like. Also compound semiconductors, such as lithium niobate, gallium arsenide and indium-phosphide, and nickel-coated mica surfaces, as used in preparation of molecules for observation in an atomic force microscope [see, e.g., Ill et al. (1993) Biophys J. 64:919] may be used as matrices. Methods for preparation of such matrix materials are well known.

For example, U.S. Patent No. 4,175,183 describes a water insoluble hydroxyalkylated cross-linked regenerated cellulose and a method for its preparation. A method of preparing the product using near stoichiometric proportions of reagents is described. Use of the product directly in gel chromatography and as an intermediate in the preparation of ion exchangers is also described.

2. Synthetic matrices

There are innumerable synthetic matrices and methods for their preparation known to those of skill in this art. Synthetic matrices are typically produced by polymerization of functional matrices, or copolymerization from two or more monomers of from a synthetic monomer and naturally occurring matrix monomer or polymer, such as agarose. Before such polymers solidify, they are contacted with the data storage device with memory, which can be cast into the material or dipped into the material. Alternatively, after preparation of particles or larger synthetic matrices, the recording device containing the data storage unit(s) can be manually inserted into the matrix material. Again, such devices can be pre-coated with glass, ceramic, silica or other suitable material.

Synthetic matrices include, but are not limited to: acrylamides, dextran-derivatives and dextran co-polymers, agarose-polyacrylamide blends, other polymers and co-polymers with various functional groups, methacrylate derivatives and co-polymers, polystyrene and polystyrene copolymers [see, e.g., Merrifield (1964) Biochemistry 3:1385-1390; Berg et al. (1990) in Innovation Perspect. Solid Phase Synth. Collect. Pap., Int. Symp., 1st, Epton, Roger (Ed), pp. 453-459; Berg et al. (1989) in Pept., Proc. Eur. Pept. Symp., 20th, Jung, G. et al. (Eds), pp. 196-198; Berg et al. (1989) J. Am. Chem. Soc. 111:8024-8026; Kent et al. (1979) Isr. J. Chem. 17:243-247; Kent et al. (1978) J. Org. Chem. 43:2845-2852; Mitchell et al. (1976) Tetrahedron Lett. 42:3795-3798; U.S. Patent No. 4,507,230; U.S. Patent No. 4,006,117; and U.S. Patent No. 5,389,449]. Methods for preparation of such matrices are well-known to those of skill in this art.

Synthetic matrices include those made from polymers and co-polymers such as polyvinylalcohols, acrylates and acrylic acids such as polyethylene-co-acrylic acid, polyethylene-co-methacrylic acid, polyethylene-co-ethylacrylate, polyethylene-co-methyl acrylate, polypropylene-co-acrylic acid, polypropylene-co-methyl-acrylic acid, polypropylene-co-ethylacrylate, polypropylene-co-methyl acrylate, polyethylene-co-vinyl acetate, polypropylene-co-vinyl acetate, and those containing acid anhydride groups such as polyethylene-co-maleic anhydride, polypropylene-co-maleic anhydride and the like. Liposomes have also been used as solid supports for affinity purifications [Powell et al. (1989) Biotechnol. Bioeng. 33:173].

For example, U.S. Patent No. 5,403,750, describes the preparation of polyurethane-based polymers. U.S. Pat. No. 4,241,537 describes a plant growth medium containing a hydrophilic polyurethane gel composition prepared from chain-extended polyols; random copolymerization is preferred with up to 50% propylene oxide units so that the prepolymer will be a liquid at room temperature. U.S. Pat. No. 3,939,123 describes lightly crosslinked polyurethane polymers of isocyanate terminated prepolymers containing poly(ethyleneoxy) glycols with up to 35% of a poly(propyleneoxy) glycol or

a poly(butyleneoxy) glycol. In producing these polymers, an organic polyamine is used as a crosslinking agent. Other matrices and preparation thereof are described in U.S. Patent Nos. 4,177,038, 4,175,183, 4,439,585, 4,485,227, 4,569,981, 5,092,992, 5,334,640, 5,328,603

U.S. Patent No. 4,162,355 describes a polymer suitable for use in affinity chromatography, which is a polymer of an aminimide and a vinyl compound having at least one pendant halo-methyl group. An amine ligand, which affords sites for binding in affinity chromatography is coupled to the polymer by reaction with a portion of the pendant halo-methyl groups and the remainder of the pendant halo-methyl groups are reacted with an amine containing a pendant hydrophilic group. A method of coating a substrate with this polymer is also described. An exemplary aminimide is 1,1-dimethyl-1-(2-hydroxyoctyl)amine methacrylimide and vinyl compound is a chloromethyl styrene.

U.S. Patent No. 4,171,412 describes specific matrices based on hydrophilic polymeric gels, preferably of a macroporous character, which carry covalently bonded D-amino acids or peptides that contain D-amino acid units. The basic support is prepared by copolymerization of hydroxyalkyl esters or hydroxyalkylamides of acrylic and methacrylic acid with crosslinking acrylate or methacrylate comonomers are modified by the reaction with diamines, aminoacids or dicarboxylic acids and the resulting carboxyterminal or aminoterminal groups are condensed with D-analogs of aminoacids or peptides. The peptide containing D-aminoacids also can be synthesized stepwise on the surface of the carrier.

U.S. Patent No. 4,178,439 describes a cationic ion exchanger and a method for preparation thereof. U.S. Patent No. 4,180,524 describes chemical syntheses on a silica support.

Immobilized Artificial Membranes [IAMs; see, e.g., U.S. Patent Nos. 4,931,498 and 4,927,879] may also be used. IAMs mimic cell membrane environments and may be used to bind molecules that preferentially

associate with cell membranes [see, e.g., Pidgeon et al. (1990) Enzyme Microb. Technol. 12:149].

3. Immobilization and activation

Numerous methods have been developed for the immobilization of proteins and other biomolecules onto solid or liquid supports [see, e.g., Mosbach (1976) Methods in Enzymology 44; Weetall (1975) Immobilized Enzymes, Antigens, Antibodies, and Peptides; and Kennedy et al. (1983) Solid Phase Biochemistry, Analytical and Synthetic Aspects, Scouten, ed., pp. 253-391; see, generally, Affinity Techniques. Enzyme Purification: Part B. Methods in Enzymology, Vol. 34, ed. W. B. Jakoby, M. Wilchek, Acad. Press, N.Y. (1974); Immobilized Biochemicals and Affinity Chromatography, Advances in Experimental Medicine and Biology, vol. 42, ed. R. Dunlap, Plenum Press, N.Y. (1974)].

Among the most commonly used methods are absorption and adsorption or covalent binding to the support, either directly or via a linker, such as the numerous disulfide linkages, thioether bonds, hindered disulfide bonds, and covalent bonds between free reactive groups, such as amine and thiol groups, known to those of skill in art [see, e.g., the PIERCE CATALOG, ImmunoTechnology Catalog & Handbook, 1992-1993, which describes the preparation of and use of such reagents and provides a commercial source for such reagents; and Wong (1993) Chemistry of Protein Conjugation and Cross Linking, CRC Press; see, also DeWitt et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:6909; Zuckermann et al. (1992) J. Am. Chem. Soc. 114:10646; Kurth et al. (1994) J. Am. Chem. Soc. 116:2661; Ellman et al. (1994) Proc. Natl. Acad. Sci. U.S.A. 91:4708; Sucholeiki (1994) Tetrahedron Lttrs. 35:7307; and Su-Sun Wang (1976) J. Org. Chem. 41:3258; Padwa et al. (1971) J. Org. Chem. 41:3550 and Vedejs et al. (1984) J. Org. Chem. 49:575, which describe photosensitive linkers]

To effect immobilization, a solution of the protein or other biomolecule is contacted with a support material such as alumina, carbon, an ion-exchange resin, cellulose, glass or a ceramic. Fluorocarbon polymers

have been used as supports to which biomolecules have been attached by adsorption [see, U.S. Pat. No. 3,843,443; Published International PCT Application WO/86 03840].

A large variety of methods are known for attaching biological molecules, including proteins and nucleic acids, molecules to solid supports [see. e.g., U.S. Patent No. 5,451,683]. For example, U.S. Patent No. 4,681,870 describes a method for introducing free amino or carboxyl groups onto a silica matrix. These groups may subsequently be covalently linked to other groups, such as a protein or other anti-ligand, in the presence of a carbodiimide. Alternatively, a silica matrix may be activated by treatment with a cyanogen halide under alkaline conditions. The anti-ligand is covalently attached to the surface upon addition to the activated surface. Another method involves modification of a polymer surface through the successive application of multiple layers of biotin, avidin and extenders [see, e.g., U.S. Patent No. 4,282,287]; other methods involve photoactivation in which a polypeptide chain is attached to a solid substrate by incorporating a light-sensitive unnatural amino acid group into the polypeptide chain and exposing the product to low-energy ultraviolet light [see, e.g., U.S. Patent No. 4,762,881]. Oligonucleotides have also been attached using a photochemically active reagents, such as a psoralen compound, and a coupling agent, which attaches the photoreagent to the substrate [see, e.g., U.S. Patent No. 4,542,102 and U.S. Patent No. 4,562,157]. Photoactivation of the photoreagent binds a nucleic acid molecule to the substrate to give a surface-bound probe.

Covalent binding of the protein or other biomolecule or organic molecule or biological particle to chemically activated solid matrix supports such as glass, synthetic polymers, and cross-linked polysaccharides is a more frequently used immobilization technique. The molecule or biological particle may be directly linked to the matrix support or linked via linker, such as a metal [see, e.g., U.S. Patent No. 4,179,402; and Smith et al. (1992) Methods: A Companion to Methods in Enz. 4:73-78]. An example of this

method is the cyanogen bromide activation of polysaccharide supports, such as agarose. The use of perfluorocarbon polymer-based supports for enzyme immobilization and affinity chromatography is described in U.S. Pat. No. 4,885,250]. In this method the biomolecule is first modified by reaction with a perfluoroalkylating agent such as perfluorooctylpropylisocyanate described in U.S. Pat. No. 4,954,444. Then, the modified protein is adsorbed onto the fluorocarbon support to effect immobilization.

The activation and use of matrices are well known and may be effected by any such known methods [see, e.g., Hermanson et al. (1992) Immobilized Affinity Ligand Techniques, Academic Press, Inc., San Diego]. For example, the coupling of the amino acids may be accomplished by techniques familiar to those in the art and provided, for example, in Stewart and Young, 1984, Solid Phase Synthesis, Second Edition, Pierce Chemical Co., Rockford.

Molecules may also be attached to matrices through kinetically inert metal ion linkages, such as Co(III), using, for example, native metal binding sites on the molecules, such as IgG binding sequences, or genetically modified proteins that bind metal ions [see, e.g., Smith et al. (1992) Methods: A Companion to Methods in Enzymology 4, 73 (1992); Ill et al. (1993) Biophys J. 64:919; Loetscher et al. (1992) J. Chromatography 595:113-199; U.S. Patent No. 5,443,816; Hale (1995) Analytical Biochem. 231:46-49].

Other suitable methods for linking molecules and biological particles to solid supports are well known to those of skill in this art [see, e.g., U.S. Patent No. 5,416,193]. These linkers include linkers that are suitable for chemically linking molecules, such as proteins and nucleic acid, to supports include, but are not limited to, disulfide bonds, thioether bonds, hindered disulfide bonds, and covalent bonds between free reactive groups, such as amine and thiol groups. These bonds can be produced using heterobifunctional reagents to produce reactive thiol groups on one or both of the moieties and then reacting the thiol groups on one moiety with

reactive thiol groups or amine groups to which reactive maleimido groups or thiol groups can be attached on the other. Other linkers include, acid cleavable linkers, such as bismaleimideothoxy propane, acid labile-transferrin conjugates and adipic acid diihydrazide, that would be cleaved in more acidic intracellular compartments; cross linkers that are cleaved upon exposure to UV or visible light and linkers, such as the various domains, such as C_H1, C_H2, and C_H3, from the constant region of human IgG_1 (see, Batra et al. (1993) Molecular Immunol. 30:379-386).

Presently preferred linkages are direct linkages effected by adsorbing the molecule or biological particle to the surface of the matrix. Other preferred linkages are photocleavable linkages that can be activated by exposure to light [see, e.g., Baldwin et al. (1995) J. Am. Chem. Soc. 117:5588; Goldmacher et al. (1992) Bioconj. Chem. 3:104-107, which linkers are herein incorporated by reference]. The photocleavable linker is selected such that the cleaving wavelength that does not damage linked moieties. Photocleavable linkers are linkers that are cleaved upon exposure to light [see, e.g., Hazum et al. (1981) in Pept., Proc. Eur. Pept. Symp., 16th, Brunfeldt, K (Ed), pp. 105-110, which describes the use of a nitrobenzyl group as a photocleavable protective group for cysteine; Yen et al. (1989) Makromol. Chem 190:69-82, which describes water soluble photocleavable copolymers, including hydroxypropylmethacrylamide copolymer, glycine copolymer, fluorescein copolymer and methylrhodamine copolymer; Goldmacher et al. (1992) Bioconj. Chem. 3:104-107, which describes a cross-linker and reagent that undergoes photolytic degradation upon exposure to near UV light (350 nm); and Senter et al. (1985) Photochem. Photobiol 42:231-237, which describes nitrobenzyloxycarbonyl chloride cross linking reagents that produce photocleavable linkages]. Other linkers include fluoride labile linkers [see, e.g., Rodolph et al. (1995) J. Am. Chem. Soc. 117:5712], and acid labile linkers [see, e.g., Kick et al. (1995) J. Med. Chem. 38:1427]. The selected linker will depend upon the particular application and, if needed, may be empirically selected.

B. **Data storage units with memory**

The data storage units are either electromagnetic memory devices or optical memory devices (OMDs), which include bar codes and other optically readable (or read/write) memories. In embodiments in which OMDs are used, the programmable devices are remote memories, such as computers into which information regarding the encoded information and linked molecules and biological particles is stored. The OMDs are read/write devices or are precoded devices.

For use with the matrices in which the memory, rather than a code, is linked to the device, any remotely programmable data storage device that can be linked to or used in proximity to the solid supports and molecules and biological particles as described herein is intended for use herein. Preferred devices are rapidly and readily programmable using penetrating electromagnetic radiation, such as radio frequency or visible light lasers, operate with relatively low power, have fast access [preferably 1 sec or less, more preferably 10^{2}-10^{3} sec], and are remotely programmable so that information can be stored or programmed and later retrieved from a distance, as permitted by the form of the electromagnetic signal used for transmission. Presently preferred devices are on the order of 1-20 mm, preferably about 1 to 10 mm, in the largest dimension and are remotely programmable using RF, microwave or radar.

Recording devices may be active, which contain a power source, such as a battery, and passive, which does not include a power source. In a passive device, which has no independent power source, the transmitter/receiver system, which transfers the data between the recording device and a host computer and which is preferably integrated on the same substrate as the memory, also supplies the power to program and retrieve the data stored in the memory. This is effected by integrating a rectifier circuit onto the substrate to convert the received signal into an operating voltage.

Alternatively, an active device can include a battery [see, e.g., U.S. Patent No. 5,442,940, U.S. Patent No. 5,350,645, U.S. Patent No. 5,212,315, U.S. Patent No. 5,029,214, U.S. Patent No. 4,960,983] to supply the power to provide an operating voltage to the memory device. When a battery is used the memory can be an EEPROM, a DRAM, or other erasable memory requiring continuous power to retain information. It may be desirable to combine the antenna/rectifier circuit combination with a battery to create a passive/active device, with the voltages supplied by each source supplementing each other. For example, the transmitted signal could provide the voltage for writing and reading, while the battery, in addition to supplementing this voltage, provides a refresh voltage for a DRAM memory so that data is retained when the transmitted signal is removed.

The remotely programmable device can be programmed sequentially to be uniquely identifiable during and after stepwise synthesis of macromolecules or before, or during, or after selection of screened molecules. In certain embodiments herein, the data storage units are information carriers in which the functions of writing data and reading the recorded data are empowered by an electromagnetic signal generated and modulated by a remote host controller. Thus, the data storage devices are inactive, except when exposed to the appropriate electromagnetic signal. In an alternative embodiment, the devices may be optically or magnetically programmable read/write devices.

1. Optically and magnetically encoded memory devices

The matrices or strips attached thereto may be encoded with a preprogrammed identifying bar code, such as an optical bar code that will be encoded on the matrix and read by laser. Such pre-coded devices may be used in embodiments in which parameters, such as location in an automated synthesizer, are monitored. The identity of a product or reactant determined by its location or path, which is monitored by reading the chip in each device and storing such information in a remote computer.

Thus, it is contemplated herein, that the memory is not proximate to the matrix, but is separate, such a memory in a remote computer or other recording device. In these embodiments, the matrices are marked with a unique code or mark of any sort. The identity of each mark is saved in the remote memory, and then, each time something is done to a molecule or biological particle linked to each matrix, the information regarding such event is recorded and associated with the coded identity. After completion of, for example, a synthetic protocol, each matrix is examined or read to identify the code. Retrieving information that from the remote memory that is stored with the identifying code will permit identification or retrieval of any other saved information regarding the matrix.

For example, simple codes, including bar codes, alphanumeric characters or other visually or identifiable codes or marks on matrices are also contemplated for use herein. When bar codes or other precoded devices are used, the information can be written to an associated but remote memory, such as a computer or even a piece of paper. The computer stores the bar code that a identifies a matrix particle or other code and information relating to the molecule or biological particle linked to the matrix or other relevant information regarding the linked materials or synthesis or assay. Instead of writing to an on-board memory, information is encoded in a remote memory that stores information regarding the precoded identity of each matrix with bar code and linked molecules or biological particles. Thus, the precoded information is associated with, for example, the identity of the linked molecule or a component thereof, or a position (such as X-Y coordinates in a grid). This information is transmitted to a memory for later retrieval. Each treatment or synthetic step that is performed on the linked molecule or biological particle is transmitted to the remote memory and associated with the precoded ID.

For example, an amino acid is linked to a matrix particle that is encoded with or marked with a bar code or even a letter such as "A" or other coded mark. The identity the amino acid linked to the matrix particle

"A" is recorded into a memory. This particle is mixed with other particles, each with a unique identifier or mark, and this mixture is then treated to a synthetic step. Each particle is individually scanned or viewed to see what mark is on each particle and the remote memory is written to describe the synthetic step, which is then associated with each unique identifier in the memory, such as the computer or piece of paper. Thus, in the remote memory the original amino acid linked to particle A is stored. After the synthetic step, the identify of the next amino acid is stored in the memory associated with "A" as is the identity of the next amino acid added. At the end of the synthesis, the history of each particle can be read by scanning the particle or visually looking at the particle and noting its bar code or mark, such as A. The remote memory is then queried to determine what amino acids are linked to the particle identified as "A" [see, e.g., Fig 20].

For example, many combinatorial libraries contain a relatively small number of discrete compounds [10^2-10^4] in a conveniently manipulable quantity, rather than millions of members in minute quantities. These small libraries are ideal for use with the methods and matrices with memories herein. They may also be used in methods in which the memory is not in proximity to the matrix, but is a remote memory, such as a computer or a table of information stored even on paper. The system depicted in FIGURE 20 is ideal for use in these methods. Polypropylene or other inert polymer, including fluoropolymers or scintillating polymers are molded into a convenient geometry and size, such an approximately 5 mm x 5 mm x 5 mm cube [or smaller or larger] with a unique identifying code imprinted, preferably permanently, on one side of each cube. If, for example, a three element code is used, based on all digits (0 to 9) and all letters of the alphabet, a collection of 46,666 unique three element codes are available for imprinting on the cubes.

The cubes are surface grafted with a selected monomer [or mixture of monomer], such as styrene. Functionalization of the resulting polymer provides a relatively large surface area for chemical syntheses and

subsequent assaying [on a single platform]. For example, a 5 x 5 x 5 mm^3 cube has a surface area of 150 mm^2, which is equivalent to about 2-5 μmol achievable loading, which is about 1-2.5 mg of compounds with a molecular weight of about 500. A simple computer program or protocol can direct split and pool during synthesis and the information regarding each building block of the linked molecules on each cube conveniently recorded in the memory [i.e., computer] at each step in the synthesis. A computer program, described below [see, e.g., Examples], or protocol can direct split and pool during synthesis and the information regarding each building block of the linked molecules on each cube conveniently recorded in the memory.

Since the cubes [herein called MACROCUBES™ or MACROBEADS™] are relatively large, they can be read by the eye or any suitable device during synthesis and the associated data can be manually entered into a computer or even written down. The cubes can include scintillant or fluorophore or label and used in any of the assay formats described herein or otherwise known to those of skill in the art.

For example, with reference to FIGURE 20, polypropylene, polyethylene or fluophore raw material [any such material described herein, particularly the Moplen resin e.g., V29G PP resin from Montell, Newark DE, a distributor for Himont, Italy] 1 is molded, preferably into a cube, preferably about 5 x 5 x 5 mm^3 and engraved, using any suitable imprinting method, with a code, preferably a three element alphanumeric code, on one side. The cube can be weighted or molded so that it all cubes will orient in the same direction. The engraved cubes 2 are then surface-grafted 3 and functionalized using methods described herein or known to those of skill in this art, to produce cubes [MACROBEADS™ or MACROCUBES™] or devices any selected geometry 4.

a. Encoded memory devices with two-dimensional bar codes and matrices with optical memories

Methods for engraving bar codes, bar codes and bar-code engraved devices are provided herein. In particular OMDs are provided and methods

for writing to the surface of these devices and reading the engraved symbology are provided. The OMDs are fabricated from a suitable material, such as black, white or colored glass, TEFLON® [polytetrafluoroethylene], polyethylene, high density polyethylene, polypropylene, polystyrene, polyester, ceramic, composites of any of these materials and other such materials. The typical OMD is 10 mm or smaller in its largest dimension and is encoded by direct deposit, dot matrix deposit, direct laser write or dot matrix scan laser write. They may be precoded or coded prior to or even during use. For use in the applications provided herein, at least one surface or a portion of a surface is treated to render it suitable for use as a support, such as by grafting, ion implant, vacuum deposit, oxidation, combiantions thereof, suitable derivatization or any other means known to those of skill in the art by which materials are treated to render them suitable for use as supports. The OMDs also have applications as a data pad for recording information about the linked molecules or for monitoring storage and location, or in clinical labs for recording relevant information. The OMDs may be in the form of microplates in which each well is encoded or in combination with any instrumentation used in biological and chemical processing and screening. The OMDs may also be fabricated as tubes, such as the MICROTUBES™ provided herein. When used with such tubular devices, they will be engraved on the outer surface, preferably the top or bottom of the device.

The material of which the OMDs are fabricated will depend upon the monitored processes. The materials that may be used include, but are not limited to, black, white or colored glass, TEFLON®, polyethylene, high density polyethylene, polypropylene, polystyrene, polyester, ceramic, such as alumina or zirconia, metal, or any composite of the above materials or any material that is physically or chemically structured to produce optical contrast as the result of exposure to the write process, which is described below. For use in the methods herein, these materials may be suitable or at least one surface there may have been treated to render them suitable for

retaining molecules and biological particles for use as matrices as described herein.

In an exemplary embodiment, illustrated in FIGURE 22, the optical memory device ["OMD"] 100 is a preferably a rectangular parallelepiped that provides a broad face upon which encoded information can be inscribed. Any geometry that is suitable for a particular application and that provides at least one surface for encoding information. The OMDs may also be containers used for chemical synthesis, such as microtiter plates, tubes, tubes adapted for use with microtiter-type plates. The two-dimensional bar code described herein is ideally suited for incorporation onto the outide surface of each well of a microtiter plate or on the outside of a small test tube or other such tube, particularly, tubes intended for use with a microplate frame, such as those available from NUNC and COSTAR. This two-dimensional bar code as wells as the method for reading and writing may also be used to track and identify other laboratory equipment, such as chromatography tubes, test tubes, beakers, flasks and other such items.

For the first exemplary embodiment of OMD 100 shown in FIGURE 22, if the OMD is formed from a ceramic material, it may have exemplary dimensions of 280 mil (L) x 140 mil (W) x 50 mil (T) [7 mm x 3.5 mm x 1.3 mm]. The dimensions of the face can be varied as needed to provide the appropriate size for recording data, providing sufficient chemical binding surface area, and to facilitate handling. The presently preferred minimum size for use with commercial feeding systems is on the order of 0.5 mm x 0.5 mm x 0.5 mm.

If the OMD 100 is formed from polypropylene or teflon, for example, it may have exemplary dimensions of 280 mil (L) x 140 mil (W) x 100 mil (T) [7 mm x 3.5 mm x 2.6 mm], although smaller dimensions are contemplated. Since OMDs made from polypropylene may be read by transmission of light through the device, the thickness must be sufficiently thin to permit transmission of light through the OMD, except where there are

darkened areas of a bar code symbol. Where reflected light is to be used, as with the ceramic OMDs, thickness need not be so limited.

For OMDs used for chemical binding or other processes for which surfaces must be specially prepared in order to assure adsorption or absorption or any means of binding of molecules or biological particles, it may be desirable to separate the binding surfaces from the data storage surface 101. In this case, one or more of sides 104 and 105, bottom 107, top 108, and back 110 may be treated to enhance binding using radiation, mechanical or chemical abrasion, or other processes as appropriate. By segregation of the binding and information surfaces, possible activation or modification of certain bound compounds by the high intensity light source used in the write process is avoided. In addition, degradation of the bar code contrast may be less on a surface that is not derivatized for binding.

If needed, segregation of the binding and information surfaces can be achieved by coating portions of the OMD with films formed from a dielectric material such as polyethylene, MYLAR, TEFLON®, KAPTON, polycarbonate, or, preferably, the para-xylylene polymers sold under the trade name Parylene [see, e.g., U.S. Patent Nos. 3,288,728, 3,342,754 and 3,429,739], or any other such materials that are commonly used in the electronics industry to passivate electronic components and circuit boards, and as a coating for medical devices, especially implants, catheters, probes and needles. [Parylene is the trade name for members of a series of polymers which are commercially available from Specialty Coating Systems, Inc., of Indianapolis, IN and originally from Union Carbide Corporation, Greenville, SC, see, U.S. Patent Nos. 3,288,728, 3,342,754 and Gorham 3,429,739; see, also brochures distributed by the manufacturer, entitled "Parylene Conformal Coatings Specifications and Properties" (© 1984, Specialty Coating Systems, Inc.), and "Parylene, A Biostable Coating for Medical Applications" (© 1984, Specialty Coating Systems, Inc.]. These

polymers provide a conformal biostable coating which electrically and chemically isolates the protected surface from its environment.

The Parylene or other such polymeric coating can be treated to form a chemically functional substrate by methods such as beta or gamma radiation, and mechanical or chemical roughening. Alternatively, polystyrene microspheres can be bonded [glued or welded] to selected surface(s) of the OMD, either on the Parylene or similar coating, or directly to the ceramic or polypropylene.

The encoded information may be stored in any optically writable and readable format. As shown printed on data storage surface 101, symbologies 106 are two-dimensional bar codes, which can be stacked rows of one-dimensional bar codes, checkerboards, or dot matrices. Other symbologies that can be used include one-dimensional bar codes, target codes, alphanumeric characters or other optically readable characters which are well known in the art. [See, e.g., Wang, et al. (1990) *A High Density Two Dimensional Bar Code* SPIE Proceedings Vol. 1384, High-Speed Inspection Architectures, Bar Coding, and Character Recognition, pp. 169-175; Martin (1991) *Unique Symbol for Marking and Tracking Very Small Semiconductor Products*, SPIE Proceedings Vol. 1598, Lasers in Microelectronic Manufacturing, pp. 206-220.]

In the exemplary embodiment, the two-dimensional bar code [e.g., symbol 106] includes an orientation indicator in the form of solid black lines across the top 120 and down the right side 122 of the symbol. Upon acquisition of the image of the symbol by the image sensing means, the image processor will utilize the orientation indicator to provide information about the rotation of the OMD relative to the sensor, and can compensate in its software by rotating the image to the appropriate orientation for decoding the image. Other types of orientation indicators as are known in the art, such as those described in the above-identified references relating to bar codes, may also be used such that physical precise orientation of the OMD within the read area is not critical. For reflection-type readers, it is only

necessary for the OMD to be right side up and the symbol is fully within the field of view of the detector, so that the symbol 106 is exposed to the image sensor. Even where reading is accomplished by transmission of light through the OMD, as in certain polypropylene embodiments, an orientation indicator in the symbol in combination with a distinctive physical or optical feature, such as described below, can provide information sufficient to determine whether the OMD is face up or face down so that appropriate compensation, such as reversal of the image, can be performed by the software in order to enable decoding.

An alternative means for recording and reading information involves the formation of a magnetic film on at least a portion of the surface of the OMD. Creation of thin magnetic films by sputtering, electroplating, or other deposition techniques are well known in magnetic recording technology. [See, e.g.,Chapter 11, "Tape and Disk Materials" from The Complete Handbook of Magnetic Recording, 3rd Edition, by Finn Jorgenson, Tab Books, 1988.] Recording and reading of data on the magnetic film can utilize conventional magnetic recording techniques.

The OMD 200 of FIGURE 23 is a variation on the embodiment of FIGURE 22 that provides a information recording section that is formed from a separate material from that of the binding surface(s) [i.e., the chemistry surface(s) or the surface(s) to which molecules or biological particles are linked]. In this embodiment, the OMD contains two sections that are linked together. Here, OMD 200 is formed from the assembly of information unit 202 and binding unit 204, with unit 202 fitting within a cavity or well 206 formed in unit 204. This embodiment provides the advantage of selecting the optimal material for each of the binding and recording processes, and also permits the information unit 202 to be assembled with the binding unit 204 after the binding unit has been treated to enhance adhesion. For example, binding unit 204 can be formed from a polymer, e.g., polypropylene, functionalized by radiation and/or chemical processes, or can be modified by bonding polystyrene microspheres to its surface(s).

Information unit 202 can be formed from plastic, ceramic or glass, and mounted within well 206 by adhesive or other bonding process, or may simply be press fit into the well. Since pre-treatment of the binding unit to enhance binding could possibly discolor the information unit, or otherwise make it less readable by modifying the surface, e.g., pitting or etching, separate formation could be advantageous. The outer dimensions of unit 202 are preferably selected to closely fit the inside dimensions of well 206 to prevent the intrusion of chemicals, or even air, into spaces between the units. In the illustrated example, unit 204 has outer dimensions of 280 mil (L) x 140 mil (W) x 100 mil (T) [7 mm x 3.5 mm x 2.6 mm] and unit 202 has maximum dimensions of 210 mil (L) x 115 mil (W) x 50 mil (T) [5.3 mm x 2.9 mm x 1.3 mm]. Since, as shown, the sides of unit 202 are beveled to form a trapezoidal cross-section to conform to a corresponding shape of the well 206, and also to assist in forming a tight seal between the two units, the actual exposed face of the information unit is on the order of 105 mil x 200 mil [2.7 mm x 5 mm]. When the two units are assembled, the combined face surface of the information and binding units are preferably flush. As can be seen, the encoded information, shown as a two-dimensional bar code symbol 208, is inscribed on information unit 202 only. With regard to the magnetic recording alternative method, the use of a separate information unit is ideal since it would generally be preferred to avoid exposure of magnetic recording media to the radiation or corrosive chemicals used for enhancement of the binding process.

Variations on the two-part OMD of FIGURE 23 are illustrated in FIGURES 25-27. In FIGURE 25, OMD 400 is illustrated where insert unit 402 is the binding unit formed, for example, from polymer functionalized by radiation and/or derivatized by suitable chemical processes or grafted to render the surface suitable for binding biological particles and molecules. Base unit 404, which may be formed from plastic, polymer, ceramic or glass, has a well 406 corresponding to the exterior shape of the binding unit 402, so that they will interfit closely. The encoded information, shown,

again, as a two-dimensional bar code symbol 412, is inscribed on the back 408 of base unit 404, opposite the face 410 at which binding unit 402 is exposed.

In FIGURE 26 [an embodiment of a microvessel], insert unit 502 has a cavity 508 covered by mesh 510 [porous material] for retaining particles but permitting chemical materials and biological particles to pass through, to form OMD 500. The chemicals pass through mesh 510 to be within cavity 508, or some material contained therein, such as microspheres, or are retained on the strands of mesh 510. As in the embodiment of FIGURE 25, base unit 504, which is encoded with the symbology, receives the binding [chemistry] unit so that it is exposed on one face 512, with the encoded information 514 located on the opposite face 516.

In the embodiment depicted in FIGURE 27, insert unit 602 is formed from polypropylene or ceramic or other suitable material and provides the information storage face 608 for writing symbology, preferably a bar code symbol 610 on OMD 600. Base unit 604 provides the means for binding of chemical materials, which contains cavity 612 which is filled with microspheres 614 and covered with polypropylene screen 616 or other suitable porous material. The base material is preferably polypropylene or other such material. In this embodiment, the information storage face 608 is on the opposite side of the OMD from the screen 616.

In yet another embodiment, OMD 700, which is illustrated in FIGURE 28, an orientation indicator is provided in the form of a notched or cut-corner 702. In this embodiment, the corner cut-out 702 will provide information as to the rotation and inversion of OMD 700, since, even if the OMD is face down, it will be apparent due to the unique outline of the face. The use of a physically detectable orientation indicator allows the handling equipment to readily detect improper positioning, for example, by placement of mechanical or optical edge detectors within the handling system. An improperly positioned OMD can be removed from the imaging position and placed back at the entry point into the reading handler, or mechanical

means, such as a retractable blade, can be provided to flip the OMD over if it is presented face down within the field of view of the reader. An alternative symbology 706 is illustrated which is, in this case, an alphanumeric code, which can be read and decoded using known optical character recognition (OCR) techniques.

Other types of orientation indicators that can be used include chamfers, holes and protrusions. Several different and distinctive shapes can be included on a single OMD to assist in orientation, positioning and separation of the OMDs. For example, a group of OMDs can have a cut corner for orientation of each OMD, with some of those OMDs having a tab extending from one of its sides, so that those with tabs can be separated from those without tabs, which facilitates division of the group for diversion to different containers.

Additional test media can be included in the OMD in the embodiment of FIGURE 29. Here, the OMD 800 has a plurality of wells or recesses 804 into which can be placed gels, beads, or the like for retaining additional chemical [molecules] or biological materials [biological particles], and/or chemical, biological or temperature sensors, or other such devices. Where such materials are placed in the wells 804, the bar code symbol 806 can include information about the nature of these materials.

The embodiment of FIGURE 30 is a variation on that of FIGURE 29. Here, the OMD 900 is partially hollow, and a plug 902 is formed in the side to permit access to the cavity 912. The front 904 and/or back 908 walls of the OMD have a mesh insert 910 which provides limited access to the cavity 912 in the OMD. A chemically- or biologically-functional material [biolgocial particle or molecule], or microspheres, for example, can be placed within the cavity 912 through plug 902 so that it is exposed to the chemical or biological materials to which the OMD is exposed without allowing direct contact between the material in the cavity and the environment in which the OMD is placed. The mesh [porous material] 910 can be polypropylene or other such suitable polymer, and of a size that makes it semi-permeable,

admitting the external solution without allowing the interior material to escape. Generally, the pore size will be within the range of 20 μm to 200 μm.

The use of OMDs and protocols therefor are flexible and can employ a variety of shapes, configurations, and polymeric synthesis supports. The ceramic 2-D bar codes will have at least 8-bytes of information content. When etched on ceramics, they are inert to the vast majority of organic synthesis conditions, easy and reliable to use, inexpensive, and very amenable to mass production. The low cross-linking polystyrene surface graft (and other polymer grafts) on the stable and inert base polymer provides an ideal solid support for chemical synthesis with excellent functionalizability and chemokinetics. This technology can also be applied to the synthesis of other types of compounds, especially small organic molecules. Among the advantages of this technology over existing combinatorial techniques are: a) low manufacturing cost; b)non-invasive encoding; c) high encoding reliability and capacity; d) total chemistry flexibility; e) excellent chemokinetics; f) easy and clean washing between reactions; g) utilization of the highly efficient directed sorting strategy; h) delivery of pure, discrete compounds in multi-milligram scale; and i) very amenable to full automation. Integration of the laser optical synthesis OMD technology with automation will further enhance its applications in high through-put chemical synthesis and biological screening.

These devices have a wide variety of applications. For example, with reference to FIG. 33E, the bar-coded OMDs devices are functionalized with amino groups to give functionalized OMDs devices **3** and used in the synthesis of oligomers. The first set of nucleosides modified with a succinic acid linker are coupled onto the matrices using DNA synthesizer with modified reaction vessels [suitable for use with the OMD-linked nascent oligonucleotides]. Five cycles of TCA de-blocking, sorting, tetrazole activation, coupling, capping, and oxidation are performed automatically on the machine [except the sorting] to yield oligonucleotides [hexamers] on the

matrices **6**. Cleavage and deprotection under standard conditions give the oligonucleotide hexamer library **7**. The identity of each oligonucleotide is associated with the unique code in a remote memory, such as by manually entry before, during or after synthesis.

b. Optically or magnetically programmed devices

In addition to electrically-programmable means for storing information on the matrix particles, optical or magnetic means may be used. One example of an optical storage means is provided in U.S. Patent No. 5,136,572, issued August 4, 1992, of Bradley, which is incorporated herein by reference. Here, an array of stabilized diode lasers emits fixed wavelengths, each laser emitting light at a different wavelength. Alternatively, a tunable diode laser or a tunable dye laser, each of which is capable of emitting light across a relatively wide band of wavelengths, may be used. The recording medium is photochemically active so that exposure to laser light of the appropriate wavelength will form spectral holes.

As illustrated In Figure 8, an optical write/read system is configured similar to that of the embodiment of Figure 7, with a vessel 212 containing a number of the particles which are separated and oriented by passing through a constricted outlet into a write/read path 206 that has an optically-transparent tube [i.e., optically transparent to the required wavelength(s)] with a cross-section which orients the particles as required to expose the memory surface to the laser 200 which is capable of emitting a plurality of discrete, stable wavelengths. Gating and detection similar to that described for the previous embodiment may be used and are not shown. Computer 202 controls the tuning of laser 200 so that it emits light at a unique wavelength to record a data point. Memory within computer 202 stores a record indicating which process step corresponds to which wavelength. For example, for process A, wavelength λ_1, e.g., 630 nm [red], for process C, λ_2, e.g., 550 nm [yellow], and for process E, λ_3, e.g., 480 nm [blue], etc. The recording medium 204 is configured to permit orientation to repeatably expose the recording side of the medium to the laser beam each time it

passes through tube 206. One possible configuration, as illustrated here, is a disc.

To write onto the recording medium 204, the laser 200 emits light of the selected wavelength to form a spectral hole in the medium. The light is focussed by lens 208 to illuminate a spot on recording medium 204. The laser power must be sufficient to form the spectral hole. For reading, the same wavelength is selected at a lower power. Only this wavelength will pass through the spectral hole, where it is detected by detector 210, which provides a signal to computer 202 indicative of the recorded wavelength. Because different wavelengths are used, multiple spectral holes can be superimposed so that the recording medium can be very small for purposes of tagging. To provide an analogy to the electrical memory embodiments, each different wavelength of light corresponds to an address, so that each laser writes one bit of data. If a large number of different steps are to performed for which each requires a unique data point, the recording media will need to be sufficiently sensitive, and the lasers well-stabilized, to vary only within a narrow band to assure that each bit recorded in the media is distinguishable. Since only a single bit of information is required to tag the particle at any given step, the creation of a single spectral hole at a specific wavelength is capable of providing all of the information needed. The host computer then makes a record associating the process performed with a particular laser wavelength.

For reading, the same wavelength laser that was used to create the spectral hole will be the only light transmitted through the hole. Since the spectral holes cannot be altered except by a laser having sufficient power to create additional holes, this type of memory is effectively non-volatile. Further, the recording medium itself does not have any operations occurring within its structure, as is the case in electrical memories, so its structure is quite simple. Since the recording medium is photochemically active, it must be well encased within an optically transmissive [to the active optical wavelength(s)], inert material to prevent reaction with the various

processing substances while still permitting the laser light to impinge upon the medium. In many cases, the photochemical recording media may be erased by exposure to broad spectrum light, allowing the memory to be reused.

Writing techniques can also include the formation of pits in the medium. To read these pits, the detector 210 with be positioned on the same side of the write/read tube 206 as the laser 200 to detect light reflected back from the medium. Other types of optical data storage and recording media may be used as are known in the art. For example, optical discs, which are typically plastic-encapsulated metals, such as aluminum, may be miniaturized, and written to and read from using conventional optical disc technology. In such a system, the miniature discs must be aligned in a planar fashion to permit writing and reading. A modification of the funnel system, described above, will include a flattened tube to insure the proper orientation. Alternatively, the discs can be magnetically oriented. Other optical recording media that may be appropriate for use in the recording devices and combinations herein include, but are not limited to, magneto-optical materials, which provide the advantage of erasability, photochromic materials, photoferroelectric materials, photoconductive electro-optic materials, all of which utilize polarized light for writing and/or reading, as is known in the art. When using any form of optical recording, however, considerations must be made to insure that the selected wavelength of light will not affect or interfere with reactions of the molecules or biological particles linked to or in proximity to matrix particles.

c. Three dimensional optical memories

3-D memory storage devices include persistant hole burning, phase holograms, and two photon optical 3-D memories that use organic materials and biomolecules. Anys such devices are intended for use herein. Of particular interest are those that use organic materials and biomolecules. Such memories can be incorporated into the matrix materials or inert polymeric materials that are derivatized for use as matrices.

d. **3-D Optical memories and apparatus therefor**

Optical memory systms are based on light-induced changes in the optical chemical or physical properties of materials. As such these memories are ideally suited for use in the methods herein and in combiation with matrices, since the materials that form the memory may be incorporated into or part of the material from which the matrix is faricated.

Polymer-based photonic materials that can store 1 trillion bytes of date per cc have been developed [see, e.g.,U.S. Patent Nos. 5,268,862, 5,130,362, 5,325,324; see, also, Dvornikov et al. (1996) Opt. Commun. 128:205-210; Dvornikov et al. (1996) Res. Chem. Intermed. 22:115-28; Dvornikov et al. (1994) Proc. SPIE-Int. Soc. Opt. Eng. 2297:447-51; Dvornikov et al. (1994) Mol. Cryst. Liq. Cryst. Sci. Technol., Sect. A 246:379-88; Dvornikov et al. (1994) J. Phys. Chem. 98:6746-52; Ford et al. (1993) Proc. SPIE-Int. Soc. Opt. 2026:604-613; Ford et al. Proc. SPIE-Int. Soc. Opt. Eng. 1853:5-13; Malkin et al. Res. Chem. Intermed. 19:159-89; Dvornikov et al. (1993) Proc. SPIE-Int. Soc. Opt. Eng. 1852:243-52; Dvornikov et al. (1992) Proc. SPIE-Int. Soc. Opt. Eng. 1662:197-204; Prasad et al. (1996) Mater. Res. Soc. Symp. Proc. 413:203-213; and Dagani in Chemical and Eng. News Sept. 23, 1996, pp. 68-69]. This technology involves using a laser to encode information in a polymeric medium containing dye molecules that have a nonliniear optical property known to those of skill in the art as two-photon absorption. When the dye molecule is irradiated with light of sufficiently high intensity, it absorbs two photons of light simultaneously; the moleucle then emits a photon of higher energy. This means that the material can be irradiated with lower energy penetrating light, such infrared or near infrared and produce a higher energy emission in the visible.

In these methods, the writing beam "photobleaches" spots in the recording medium so that those spots when subsequently illuminated with a reading beam will emit either no light or less light than the surrounding medium [see, e.g., U.S. Patent No. 5,325,324; see, also U.S. Patent No.

5,130,362]. By varying the intensity of the writing laser, the extent of photobleaching can be varied to get a gray-scale, thereby permitting storage of inromation in analog as well as digital form. Dyes have been developed that are particularly suitable for use in these methods. For example, a stilbene derivative with sbustituted amino and sulfonyl groups [APSS] has been developed that has very strong 2-photon absorption. The dye is dispered in a polymer, such as a methacrylate polymer, which then serves as a read/write medium. Memories based on phtochromaic materials, such as 1-nitro-2-naphthaldehyde and the colorless base form of the laser dye rhodamine B, are also available [see, e.g., Dvornikov et al. (1996) Res. Chem. Intermed. 22:115-28].

As noted above, incorporation of the these dyes or other such molecules into the polyermic supports used in the syntheses and assays described herein will permit the supports [or portions thereof] to serve as memories to which information can be written and from which it can be read. Thus, for example, instead of using a symbology as described in the embodiments herein, the polymer from which support is made will contain a dye molecule or other molecule that exhibits 2-photon absorption, and thereby serve as a storage medium that can be read or can be a read/write medium. The other portion of the device can be radiation grafted and used as a support for chemical syntheses and assays.

e. Reading and writing to matrices with optical memories

An exemplary read/write system is illustrated in FIGURE 24. The write system includes laser 320, mirror 322, and prism 324 mounted on drive shaft 325 connected at a first end to drive motor 326. Drive shaft 325 is connected at its second end to geared linkage 328 which rotates drive shaft 329 and prism 330 in synchrony with prism 324. The beam emitted by laser 320 follows optical path 310 to mirror 322, where it is reflected toward prism 324. Prism 324 rotates to scan the beam along the y-axis, i.e., up and down, so that the beam effectively shifts top to bottom

by reflection from the prism faces in succession as it rotates. This beam is similarly scanned along the x-axis from left to right by reflection from the faces of prism 330 in succession as prism 330 rotates. Either or both prisms can be replaced with rotating or oscillating mirrors to achieve the same scanning pattern, in a manner similar to the scanning mechanisms used in conventional laser-based bar code scanners. See, e.g., U.S. Patent No. 4,387,297 to Swartz, et al., entitled "Portable Laser Scanning System and Scanning Methods", and U. S. Patent No. 4,409,470 to Shepard, et al., entitled "Narrow Bodied, Single- and Twin-Windowed Portable Laser Scanning Head for Reading Bar Code Symbols", the disclosures of which are incorporated herein by reference. Mirrors 332 and 334 provide means for directing the beam toward the OMD 300 at the appropriate level, and, thus, are positioned in consideration of the guide means, so that the beam impinges upon the desired recording surface.

As illustrated in FIGURE 24, OMD 300 has already been inscribed during an earlier process step, evidenced by the fact that symbology 306 is present and complete. Also as illustrated, symbology 316 is currently being written by the progression of laser spot 336 across the write surface, as scanned by prisms 324 and 330. The contrasting dark and light areas of the symbologies 306,316 are created by pulsing the laser 320 according to a signal provided by system controller 338.

In an exemplary embodiment, laser 320 is a CO_2 laser, which emits light in the infrared at a wavelength of 10.6 μm. The writing process is accomplished by using a sufficiently high power beam to burn the surface of the OMD, formed of ceramic, white polypropylene or the like, to produce a dark carbon build-up corresponding to the dark lines of the symbology on the lighter colored background. The exemplary laser power is 25 W, with a spot size of 0.03 mm, burning a dot in the write surface of 0.13 mm, to create a two-dimensional bar code using a dot matrix pattern. Mirrors 322, 332 and 334, and prisms 324 and 330 must be coated with an appropriate IR-reflective film to avoid damage to the optics by the laser. In read

systems which utilize transmission of light through the OMD, the carbon build-up will block the light, appearing as darkened areas to a sensor on the opposite side of the OMD from the light source. In read systems which utilize back-reflection, the carbon build-up will absorb light, while the other areas will reflect the light, again creating a contrast between the inscribed and untouched areas of the surface.

Since the IR write beam is not visible, it may be desirable to use an optically-visible laser 336, for example, He-Ne or a diode laser which emits within the visible spectrum, or other focussed light source, to emit a beam along optical path 310 to permit visual alignment. Optically-visible laser 339 may also be used as a proximity detector to send a signal to the system controller to indicate the presence of the OMD in the write position to trigger the write process, either by reflection or by blocked transmission using conventional optical sensors. Alternatively, a separate optical detector system may also be used to detect and indicate the presence of an OMD in the write or read position.

For OMDs formed from glass or ceramic, a beam from a CO_2 laser can be used to etch the glass to produce contrasting lines by modifying the surface finish of the glass. For example, the glass can be frosted or otherwise roughened, which may assist in the binding of compounds, and which has a reduced reflectivity. Upon exposure to the high power write beam, the glass surface is partly flowed, i.e., partly melted, so that a smooth, highly reflective surface remains after the surface cools. Contrast between the frosted and flowed glass can be enhanced for reading by selecting a read wavelength which maximizes the differences in reflectivity between the two surface finishes.

Other types of lasers which may be used include neodymium-YAG [yttrium-aluminum-garnet], excimer, or any other laser capable of emitting a sufficiently high power beam to modify the material surface to produce an optically-readable contrast. Alternative lasers for the writing process include diode lasers, such as those made by Coherent, Inc. of Santa Barbara, CA.

Among those that are suitable are Model No. S-98-2000C-200-C, T or H, which emit light at 980 nm with a CW power of 2000 mW, Model No. B1-81-10C-19-30-A or W, which emit light at 808 nm with a CW power of 10,000 mW, or Model No. B1-81-15C-19-30-A or W, which emit light at 808 nm with a CW power of 15,000 mW.

Selection of the laser will depend on the material of which the OMDs are formed. For example, if an optically-reactive material is encased within a transparent glass or plastic shell, any laser capable of inducing the readable change in the optically-reactive material would be acceptable. Photochemically-reactive media, such as that disclosed for the "Optical Data Storage System" which is the subject of U.S. Patent No. 5,136,572 to Bradley, incorporated herein by reference, can be selectively activated and read by use of wavelength tunable diode lasers, such that a lesser power and/or visible light laser can be used with a more reactive recording media.

The range of movement of the laser spot 336 is limited, generally to the area of the write surface, so that the OMDs must be moved past a target area within which laser spot 336 is projected. Movement of the OMDs can be achieved by one or more sets of conveyor belts 370, chutes or guide rollers, each of which can be fed by a commercial-type centrifugal feeder, such as those available from Hoppmann Corporation of Chantilly, VA and Kirchlintein, Germany. Feeders of this type are known in industry for mass handling of parts and products, including foods, pharmaceuticals, containers and hardware. Linear and vibratory feeders are also known and may be used for handling the OMDs. An exemplary handling system is illustrated in FIGURE 32 and will be discussed in more detail below.

Included in the proximity location process to detect the presence of an OMD within the write position can be a detector 372 for locating the next available area on the write surface for writing. In the example illustrated in FIGURE 24, for a write surface having four available locations, position #1 is already filled with symbology 306, and position #2 is in the process of being filled with symbology 316. A light source 368, such as a

visible laser, emits a beam which is directed by scanning optics 370 toward the write surface of the OMD. As the write surface is scanned by the beam, the next available area detector 372 can look sequentially at each OMD as its presence is detected, first at position #1, then at subsequent positions until it finds an area on which no symbology is written, i.e., no contrasting markings are detected, or a "white" area of a pre-determined width is detected which is wider than the "quiet Zone" which is commonly included in bar codes. [See, e.g., Wang, et al., *"A High Density Two Dimensional Bar Code,"* SPIE Proceedings Vol. 1384, High-Speed Inspection Architectures, Bar Coding, and Character Recognition (1990) pp. 169-175.] This location process permits multiple uses of OMDs, and takes into consideration that some OMDs may be exposed to a greater number of process steps than others before being combined into the string in which they are presently included.

The detector 372 can also be used for indication of the presence of OMDs to be read. In the case of reading, the detector 372 can also be used to identify the presence of all symbologies to be scanned for reading, which is particularly important if a laser beam or other relatively narrow beam of light is scanned over the written surface to read the symbology. Where an incoherent light source is used to simply flood the entire write surface with light, such as a lamp 340, the ability to detect the presence of individual symbologies is not critical, since the entire write surface will be viewed and recorded at once using frame grabbing techniques.

During the read process, after the presence of an OMD is indicated, the lamp 340 is activated to illuminate the write surface. The light reflected from the surface is modulated by the symbology printed thereon due to the selective reflection and absorption of the contrasting areas. Optics 342, which will typically be an assembly of lenses and filters, which remove stray light, focus the reflected light onto detector 344. Selection of optics can be performed in a manner similar to that disclosed in U.S. Patent No. 5,354,977 of Roustaei, incorporated herein by reference, which describes

an optical bar code scanner using a CCD [charge-coupled device] detector and associated optics. In the same reference, a detailed description of CCD detectors for use in bar code scanners is provided, as well as steps for processing the signal generated by the CCD detector.

In the exemplary embodiment illustrated in FIGURE 24, the CCD detector 344 contains an array of discrete devices, each of which is a "pixel", capable of storing charge impinging upon it representative of reflected light from the write surface, then reading out the charge as a serial analog waveform. A typical CCD array for bar code scanning has 2048 pixels, however, CCD arrays of other dimensions may be used. In the preferred embodiment, a CCD array of 640x480 pixels is used. Using the CCD array, a "snap shot" of the OMD surface is created using known image or frame grabbing techniques, and an analog electrical representative of the snap shot is conducted to the signal processing function 348 within the system controller, which includes an analog-to-digital converter, to convert the signal into an output of the data written on the OMD.

In a preferred embodiment, the detector is a commercially-available, PC-interfaceable CCD camera sold under the trademark QuickCam™ by Connectix Corporation of San Mateo, California, which has a resolution of 640 x 480 pixels. Any other such camera may be used. The camera has a manually-adjustable focus lens, but image acquisition is otherwise controlled by the system controller 348, which, as part of its software, initializes the camera for frame grabbing. Any such PC-interfaceable CCD camera with a similar or better resolution may be used. For example, other types of detector arrays are known within the bar code scanning technology, including CMOS sensors, such as described in the article entitled "CMOS in camera", *IEE Review*, May 1994, p. 111, incorporated by reference, which are also capable of generating "snap shots" of the data written on the OMD and could be used in place of the CCD detector array 344.

Processing of the image grabbed by the image detector is a significant aspect of the system in that it provides the flexibility to

manipulate the image to enhance readability. The steps of the exemplary image processor are provided in the flow diagram of FIGURE 31, and the image signal generated by the detector is checked for completeness, validity and orientation, among other things. As discussed above, if systems where physical orientation and positioning of the OMD is not assured by the handling hardware, one aspect of the image processing software is to determine skew or rotation of the image as seen by the detector.

The following steps are provided in detail in the system processor's software, of which a portion is depicted in the flow diagram of FIGURE 31. [Note that the actual image obtained from the camera can be displayed on a system monitor as it is being modified to permit decoding.] First, after obtaining the image from the camera [step 1001], in steps 1002 and 1003, the edges of the symbol in the vertical direction are identified, looking for the highest peak signal to provide a reference, then the horizontal edges are found [step 1004]. Knowing the boundaries of the symbol, the reasonable spacing is determined [step 1005] to correct for missing or extra vertical edges using a neural network approach. Based on the reasonable spacing, it is determined if the length of the vertical edge is appropriate [step 1006]; if not, adjustments are made by adding or removing edges [step 1007]. A similar procedure is used for the horizontal edges [steps 1008-1010], allowing skew to be determined. Having determined the orientation and spacing of the symbol, the symbol is broken into sections [step 1011], or cells, and the average intensity for each cell is determined [step 1012] to permit calculation of the threshold [step 1013] for distinguishing a dark from a light area of the code. Following this, parity checks are performed [step 1014] to provide an indication of whether the complete symbol was detected, i.e., the correct number of bits was obtained, or whether the signal was corrupted. In the preferred embodiment, 17 bits of the data contained within the two-dimensional bar code are dedicated to parity checking. At this point, if a corrupted signal is indicated, and the error is not corrected [step 1015], rather than proceeding with an attempt to

decode, the image processor initiates a new scan of the symbol [step 1001]. If the signal is good, the cells are converted to code [step 1016] which is decoded [step 1017].

The image processing system converts the stored image into a series of rows [and columns for two-dimensional codes] containing binary data, with dark lines indicating highs, or ones, and white lines indicating lows, or zeroes [or vice versa]. To enhance accuracy, a number of processing steps may be performed, and the resulting data can be the average of all processing steps for that particular image.

Although efforts are taken initially to avoid modification of the surface on which the information is written, because each OMD is presumably being subjected to a significant number of process steps, the appearance of the symbol may degrade with time due to accumulation of chemicals or surface roughening, resulting in decreased contrast between the light and dark lines of the code. This issue can be addressed with software, which can compensate for the deterioration of the symbol. In a preferred embodiment, the software includes neural network algorithms which can be trained to learn the specific cumulative effects of chemical processing and compensate by either recalibrating the detector, for example, to increase the exposure time, to modify the illumination, to increase the number of verification decode steps used for averaging, or to adjust the threshold between "dark" and "light". Two appropriate commercially-available neural network programs are Thinks™ and ThinksPro™ [published by Logical Designs Consulting, Inc. La Jolla, CA; see, U.S. Patent No. 5,371,809], which are designed to run on personal computers, and provides numerous different and well known training algorithms and methods. Other neural network software products are commercially available, including Neural Works Professional™ by NeuralWare, NeuroShell2™ by Ward Systems, BrainMaker Professional™ by California Scientific, and Neural Net Tool Kit™ by Math Works. Each could be used for training of the signal processor to compensate for degraded contrast in the symbol, and selection of the

appropriate program or creation of an appropriate program would be within the level of skill in the art.

FIGURE 32 provides a diagram of an exemplary handling system for separating and reading and/or writing to an OMD, particularly those in the shape of a parallelopiped. Such handlers are commercially avaiable [e.g., from Hoppmann Corporation, Chantilly, VA, see, U.S. Patent Nos. 5,333,716, 5,236,077, 5,145,051, 4,848,559 4,828,100, 4,821,920, 4,723,661 and 4,305,496]. The OMDs are placed in vibratory feeder 1102 by way of supply hopper 1104. Vibratory feeder 1102 includes rings and ramps [not shown] which support the OMDs as they move within the feeder, driven by the feeder's vibration in a direction toward exit chute 1106. An orientation rim, bar, or other feature [not shown] may be included in the internal ramps or exit chute to rotate the OMDs when a physical orientation indicator, such as the cut corner, is provided. Exit chute 1106 feeds the OMDs to ramp 1110 of linear feeder 1108. The reciprocating motion of the ramp 1110 causes the OMDs to move forward [to the left in the figure] toward walking beam 1112 and within the field of view of camera 1114. [Where a write operation is to be performed, the write laser and optics can be positioned in place of or nearby the camera.] Movement of the walking beam 1112 is stepped so as to pause advance motion of the OMD to allow writing and/or reading of the appropriate information.

After completion of the writing or reading step, the OMD is advanced along the walking beam 1112 toward one or more vials or flasks 1114 containing chemical or biological solutions. Ramps [not shown] leading from the walking beam to the vials or flasks 1114 can be selected by opening gates, or by tilting the walking beam 1112 in front of the selected vial, thus feeding the OMD into the desired vial for the next process step. The vials or flasks 1114 can be fixed within a tray or rack that allows it to be removed after the processing has finished so that the OMDs can be dumped into the hopper of the same or another feeder to repeat the above steps for handling, writing, reading, and distributing the OMDs to the next process step.

It may be desirable to include a protective enclosure 1116, such as a polycarbonate and polyphenylene oxide resins, preferably the polycarbonate resin sold under the name LEXAN™ [the well known polycarbonate resin commercially available from General Electric Corp, Waterford, NY, or MERLON™ made by Mobey Chemical Co., Pittsburg, PA] or the resin sold under the trademan NORYL [from General Electric Corp] other such polymer such as polyethylene, lucite, bakelite and other such resins that have high tensile and impact strength over a broad temperature range, are virtually shatter-proof and are extrudable as transparent sheets, over the handling system to prevent contamination of the OMDs and solutions as well as for the safety of the system operator.

2. Electromagnetically programmable devices

The programmable devices intended for use herein, include any device that can record or store data. A preferred device will be remotely programmable and will be small, typically on the order of 10-20 mm^3 [or 10-20 mm in its largest dimension] or, preferably smaller. Any means for remote programming and data storage, including semiconductors and optical storage media are intended for use herein. These include Yagi antennaes [see, e.g., Roland et al. (1996) Nature 381:120], diodes, magnetic tapes, and any other medium for storing information.

Also intended for use herein, are commercially available precoded devices, such as identification and tracking devices for animals and merchandise, such those used with and as security systems [see, e.g., U.S. Patent Nos. 4,652,528, 5,044,623, 5,099,226, 5,218,343, 5,323,704, 4,333,072, 4,321,069, 4,318,658, 5,121,748, 5,214,409, 5,235,326, 5,257,011 and 5,266,926], and devices used to tag animals. These devices may also be programmable using an RF signal. These device can be modified, such as by folding it, to change geometry to render them more suitable for use in the methods herein. Of particular interest herein are devices sold by BioMedic Data Systems, Inc, NJ [see, e.g.,the IPTT-100 purchased from BioMedic Data Systems, Inc., Maywood, NJ; see, also U.S.

Patent Nos. 5,422, 636, 5,420,579, 5,262,772, 5,252,962, 5,250,962, and see, also, U.S. application Serial No. 08/322,644, filed October 13, 1994]. ID tags available from IDTAG™ Inc, particularly the IDT150 read/write transponder and the IDT101 [IDTAG™ Ltd. Bracknell, Berks RG12 3XQ, UK, fabricated using standard procedures and the method for coil winding, bonding and packaging described in International PCT application Nos. WO95/33246, WO95/16270, WO94/24642, WO93/12513, WO92/15105, WO91/16718; see, also U.S. Patent Nos. 5,223,851 and 5,281,855] are also preferred herein. The IDT150 is a CMOS device that provides a kilobit of EEROM. This transponder also includes a 32 bit fixed code serial number that uniquely identifies each chip. The IDTAG™ transponder transmits data to a transceiver system by amplitude modulating its coil and generating an EM field. It receives data and commands from a transceiver by demodulating the field received by the coil and decoding the commands. The transponder derives its power source from a frequency emitted in the signal from the reader, to which the transponder emits a response. A smaller version [that has 16 bit EEROM] and is about 11 mm x 4 mm x 3 mm of this transponder is also among preferred devices. These transponders are packaged in glass or polystyrene or other such material. Also preferred herein, are tags fabricated by and available from MIKRON under the name HITAG® [see, U.S. Patent No. 5,345,231 for a description of the systems for reading and writing].

In a preferred embodiment herein, the data storage unit includes a semiconductor chip with integrated circuits formed thereon including a memory and its supporting circuitry. These devices can be written to and interrogated from a distance. A radio frequency transmitter/receiver system supplies power to program and retrieve data. In particular, the data storage unit preferably includes a programmable read only semiconductor memory [PROM], preferably a non-volatile memory or other memory that can store data for future retrieval, that will have information describing or identifying the molecules or biological particles linked to or in proximity to the matrix.

This information either identifies the molecule or biological particles including a phage and viral particles, bacteria, cells and fragments thereof, provides a history of the synthesis of the molecule, or provides information, such as a batch number, quality control data, reaction number, and/or identity of the linked entity. The memory is programmed, before, during or, preferably, after, each step of synthesis and can thereafter be read, thereby identifying the molecule or its components and order of addition, or process of synthesis.

While many well known read only memory devices use fuse structures that are selectively "blown" to store data points, with a fuse located at each possible data address in an array, among the devices of interest herein are those that rely on antifuse programming technology, in which short circuits are selectively created through an insulating layer separating word and bit lines in an array. Due to the relatively low level of voltage supplied by the transmitted signal when the memory device is passive, antifuse memories are readily used because of the lower voltage requirements for writing.

Thus, suitable memory devices, are about 1-20 mm in the smallest dimension [or smaller], are rapidly programmable [1 sec, preferably 1 msec or less], can be interrogated from a distance [distances of about a centimenter up to about an inch are presently preferred], and are programmable using electromagnetic radiation, preferably frequencies, such as those within the radio frequency range, that do not alter the assessed activities and physical properties of the molecules and biological particles of interest.

Devices that rely on other programmable volatile memories are also intended for use herein. For example, a battery may be used as to supply the power to provide an operating voltage to the memory device. When a battery is used the memory can be an EEPROM, a DRAM, or other erasable memory requiring continuous power to retain information. It may be

advantageous to combine the antenna/rectifier circuitry with a battery to create a passive/active device, in which the voltages supplied by each source supplement each other. For example, the transmitted signal could provide the voltage for writing and reading, while the battery, in addition to supplementing this write/read voltage, provides a refresh voltage for a DRAM memory so that data is retained when the transmitted signal is removed. A 2 mm x 2 mm x 0.1 mm chip [or 3 mm x 3 mm x 0.1 mm] is presently among the preferred chips [fabricated by Sokymat]. This chip has monolithic antenna. Such chips addressable [read/write] using microwave [Ghrtz] range frequencies.

a. Antifuses

An antifuse contains a layer of antifuse material sandwiched between two conductive electrodes. The antifuse device is initially an open circuited device in its unprogrammed state and can be irreversibly converted into an essentially short circuited device by the application of a programming voltage across the two electrodes to disrupt the antifuse material and create a low resistance current path between the two electrodes.

An exemplary antifuse structure for use herein is formed by defining a word line of heavily N-doped polysilicon on an insulating substrate, depositing an antifuse layer of lightly N-doped semiconductor over the polysilicon, and defining a metal address [or bit] line upon and in electrical contact with the antifuse layer. The semiconductor material used for the antifuse layer is typically selected from among silicon, germanium, carbon and alpha-tin. The properties of the semiconductor material are such that the material is essentially non-conductive as long as the voltage across it does not exceed a threshold level. Once the threshold voltage is exceeded, a conductive filament is formed through the semiconductor so that the resistance between the metal and polysilicon lines at the points at which they cross irreversibly switches from a high resistance state to a relatively low resistance state.

To program or change the resistance of the antifuse from a very high level [greater than 100,000,000 ohms] to a low level [less than 1000 ohms], a voltage of sufficiently high electrical field strength is placed across the antifuse film to create a short circuit. The voltage level required to induce breakdown is determined by the level of dopant in the antifuse layer. As breakdown occurs electrical current will flow through one small region of the film. The current is limited by the resistance of the filament itself as well as any series resistance of conductive layers or logic devices [transistors] in series with the antifuse.

Examples of the antifuse and its use as a memory cell within a Read-Only Memory are discussed in Roesner et al., "Apparatus and Method of Use of Radio frequency Identification Tags", U.S. application Serial No. 08/379,923, filed January 27, 1995, Roesner, "Method of Fabricating a High Density Programmable Read-Only Memory", U.S. Pat. No. 4,796,074 (1989) and Roesner, "Electrically Programmable Read-Only Memory Stacked above a Semiconductor Substrate", U.S. Pat. No. 4,442,507 (1984). A preferred antifuse is described in U.S. Patent No. 5,095,362. " Method for reducing resistance for programmed antifuse" (1992) [see, also U.S. Patent No. 5,412,593 and 5,384,481].

U.S. Patent No. 5,095,362 provides a method for fabricating a layer of programmable material within an antifuse that exhibits relatively lower than normal resistance in its programmed state and also provides a semiconductor device containing an antifuse film of the type composed of semiconductor material having a first electrical state that is characterized by high electrical resistivity and a second electrical state that is characterized by low electrical resistivity.

The means for selectively decreasing resistivity includes nonactivated conductive dopants that are ion implanted within the otherwise highly resistive semiconductor material. The dopants as implanted are in a nonactivated state so that the dopants do not enhance the conduction of carriers in the film. Once activated, the dopants enhance the conduction of

carriers in the film. Activation of the dopants occurs upon application of a threshold voltage across a predetermined and selected portion of the material in which the dopants are disposed. The selected portion is defined by the crossover point of selected word and bit [or address] lines. The dopants are N-type, selected from among antimony, phosphorous, arsenic, and others to provide additional charge carriers. The implant dosage is used to determine the threshold voltage level that will be required to induce formation of the conductive filament. P-type dopants, such as boron, may also be used to affect a change in programming voltage.

b. A recording device with non-volatile, such as anti-fuse-based, memory

Figure 5 depicts a recording device containing a non-volatile electrically-programmable read-only memory [ROM] 102 that utilizes antifuse technology [or EEPROM or other suitable memory] is combined on a single substrate 100 with a thin-film planar antenna 110 for receiving/transmitting an RF signal 104, a rectifier 112 for deriving a voltage from a received radio frequency [RF] signal, an analog-to-digital converter [ADC] 114 for converting the voltage into a digital signal for storage of data in the memory, and a digital-to-analog converter [DAC] 116 for converting the digital data into a voltage signal for transmission back to the host computer is provided. A single substrate 100 is preferred to provide the smallest possible chip, and to facilitate encapsulation of the chip with a protective, polymer shell [or shell + matrix or matrix material] 90. Shell 90 must be non-reactive with and impervious to the various processes that the recording device is being used to track in order to assure the integrity of the memory device components on the chip. Materials for the shell include any such materials that are known to those of skill in the art [see, e.g., Hiroshi et.al., eds. (1995) Polymeric Materials for Microelectronic Applications: Science and Technology, ACS Symposium Series No. 579], including glasses, ceramics, plastics and other inert coatings.

Based on current semiconductor integrated circuit fabrication process capabilities, in a preferred embodiment the finished chip on which all of the listed components are integrated is on the order of 1 mm x 1 mm [~ 40 mils x 40 mils], with a memory capacity of about 1024 bits, but can have greater or lesser capacity as required or desired. Greater memory capacity, where needed, and smaller chips, however, will be preferred. The chip may be larger to accommodate more memory if desired, or may be smaller as design rules permit smaller transistors and higher device densities, i.e., greater memory capacity.

The antifuse ROM structure described herein, and the method for fabricating the same, are based upon the teachings of United States Patent No. 4,424,579, issued January 3, 1984, No. 4,442,507, issued April 10, 1984, No. 4,796,074, issued January 3, 1989, and No. 5,095,362, issued March 10, 1992, all of Roesner, No. 4,598,386, issued July 1, 1986, of Roesner et al., and No. 5,148,256, issued September 15, 1992 and No. 5,296,722, issued March 22, 1994, both of Potash, et al., and also U.S. application Serial No. 08/379,923, filed January 27, 1995, to Roesner et al., all of which are incorporated herein by reference.

In an antifuse-type memory device, the individual memory cells are arranged in arrays of orthogonal conductive word and bit lines to obtain the smallest possible memory array size. For example, for 1024 bits of memory, there are 32 word lines and 32 bit lines for a square array. Memories with greater capacity may also be used. Schottky diodes are formed generally corresponding to the points at which the word and bit lines cross. The word and bit lines are separated by an undoped or lightly-doped semiconductor layer with interstitial doping. The semiconductor layer may also be amorphous silicon with implanted dopants in a nonactivated state. Each of these crossover points is a memory cell and is the equivalent of a programmable switch in series with a Schottky diode. Data are stored by the switch being ON or OFF. As fabricated, an antifuse memory device has all of its switches in the OFF state. A switch is turned on by applying a

voltage in excess of a pre-determined threshold voltage to one of the word lines while setting a selected bit line to a low logic level. The threshold voltage is determined by the impedance of the semiconductor layer, i.e., its doping level. According to the process for fabricating the antifuse memory of the preferred embodiment, the impedance can be less than 200 ohms with a threshold voltage for programming as low as 3 volts. Since in the embodiment described herein the programming voltage is provided solely by the rectified RF signal, a low threshold is preferred. Application of voltage exceeding the threshold activates the interstitial dopant in the semiconducting film at the point corresponding to the cross-over between the two lines, causing a short between the word and bit lines and irreversibly turning on that particular switch or memory cell. Address decoders, as are known in the art, are used to selectively address the word and bit lines for purposes of both writing information to and reading stored information from the memory array. [See, e.g., U.S. Patent No. 5,033,623, 5,099,226, 5,105,190, 5,218,343, 5,323,704]. Exemplary means for decoding information to be stored in memory and to be read from memory are provided in Patents No. 4,442,507 and No. 4,598,386.

Information to be written into the memory need not be detailed since the data stored in the memory is primarily acting as an identification marker that is traceable to a more detailed record stored in the host computer memory 120, independent of the memory associated with the matrix support or tagged molecule or biological particle. In this manner, the RF signal from transmitter 80 that is used to provide the power and the signal to the matrix particle memory need only address a single memory cell to indicate that a nascent oligomer linked to or in proximity to the memory device has been subjected to a given process step or to identify a molecule or biological particle. In other words, a conventional "push-pull" type of address decoder, where only one bit line and one word line are driven high and low, respectively, at any given time, may be used. Thus, a sophisticated memory addressing system need not be provided on the matrix particle

memory chip, and shift registers may be used to control memory addressing. Alternatively, a microprocessor which is mask-programmed during the fabrication process for controlling an address bus which connects the ADC 114 and the DAC 116 to the memory array may also be built onto the same substrate on which the memory and other components are integrated. Other integrated means for selectively addressing locations within the memory are known and will be apparent to the practitioner skilled in the art.

As described above, antifuse memories are well known in the art. These memories include structures in which the word and bit lines may both be made of either N+ polysilicon or metal [aluminum or aluminum-silicon], separated by silicon dioxide (SiO_2), silicon nitride (Si_3N_4), combinations thereof, or amorphous silicon alone or in combination with SiO_2 and/or Si_3N_4. In each case, a short circuit is created at locations in the antifuse material corresponding to the crossover location of selected word and bit lines by applying a voltage in excess of a pre-determined threshold voltage.

Examples of alternate means for forming an antifuse memory are provided in the following U.S. Patents: No. 5,248,632, issued September 28, 1993, of Tung et al.; No. 5,250,459, issued October 5, 1993, of Lee, No. 5,282,158, issued January 25, 1994, of Lee; No. 5,290,734, issued March 1, 1994, of Boardman, et al.; No. 5,300,456, issued April 5, 1994, of Tigelaar et al.; No. 5,311,039, issued May 10, 1994, of Kimura, et al.; No. 5,316,971, issued May 31, 1994, of Chiang et al.; No. 5,322,812, issued June 21, 1994, of Dixit, et al.; No. 5,334,880, issued August 2, 1994, of Abadeer, et al., and others.

Generally for use in the methods herein, non-volatility of the memory or the ability to lock or prevent erasure is preferred since power is applied to the chip only when it is subjected to the RF or other transmission signal for reading or reading and writing. Further considerations are the voltage levels required for writing into memory, since the threshold voltage must be less than the maximum voltage of the rectified RF signal in order to assure that sufficient voltage is always available during the writing process. The write

voltage may be enhanced by supplementing the RF-supplied voltage with optically-generated voltage, such as a photocell. Photocells on semiconductor substrates are well known in the art and could be easily integrated onto the chip. A laser or other light source could be readily included in the write apparatus to illuminate the chip coincident with transmission of the RF write signal. Similarly, other forms of electromagnetic radiation may be used to provide additional power, if needed.

Although antifuse memories are not designed to be erasable, it may be desirable to re-use the devices if the memory becomes full. In such instances, conventional electrically programmable erasable read only memories (EEPROMs) may be used instead. Since EEPROMs require higher write voltage levels, it may be desirable to supplement the RF-supplied voltage as described above. In EEPROMs, stored data can be erased by exposing the device to UV light.

Signal rectifier 112 may be one or more Schottky diode(s), making it readily incorporated into the fabrication process used for the memory array. Other means for signal rectification may be used as are known. The ADC 114 and DAC 116 are well-known devices and are readily integrated onto the substrate 100 using the fabrication process described in the references for the memory array. Radio frequency modulation techniques, which are known in the art, for example, pulse code modulation, may be adapted to permit direct digital transmission, in which case the ADC and DAC may not be required.

Antenna 110 is formed during the fabrication process using conventional photolithographic techniques to provide one or more metal structures, such as aluminum, to receive a pre-determined wavelength RF transmission. The antenna may be a simple straight line half-wave antenna which is created by patterning a structure during the second metal process steps so that the structure has a length equal to one-half of the wavelength of the selected RF transmission frequency in free space. Another option for

formation of the antenna is as a small loop, either on a dedicated portion of the chip, or encircling the other components of the chip, also formed during the second metal step of the fabrication process. It is noted that, in a typical semiconductor fabrication process, such as would be compatible with the preferred antifuse memory, the first and second metal steps include depositing a layer of aluminum, then patterning the aluminum photolithographically followed by a plasma etch to define the desired features. Except where vias are formed, the two metal layers are separated by a dielectric film. Dipole antennas may be formed by patterning the second metal in a similar manner, with the dimensions of the antenna being selected for the appropriate RF frequency. The two metal layers may also be used to form a microstrip antenna structure by selecting the dielectric film between the metal layers such that it has a dielectric constant and thickness appropriate so that the microstrip is resonant at one-half of the RF wavelength. [The first metal layer provides the ground plane.] The metal structures, which may be square patches, circles, lines, or other geometries, are defined photolithographically during the normal masking steps of the first and second metal processes. Other antenna structures which can be configured as a thin film device for integration onto a common substrate with the memory structure and other components may be used and will be apparent to those skilled in the art. Similarly, a resonant circuit [inductor-capacitor] can be readily integrated onto the chip, with the resonant circuit being tuned to the RF carrier signal of the transmitter.

Frequency tuning of either an antenna or resonant circuit can provide additional coding capability. For example, a first group of memory devices can be tuned to receive a carrier wave of a first RF frequency, e.g., f_1, and a second group could be tuned to receive a second frequency f_2, and so on. The separate carrier frequencies could provide additional means for tracking or providing information to the devices, even if the groups become intermixed.

The RF antenna may, in an alternate embodiment, be formed external to the semiconductor substrate. In this configuration, a separate conductive wire, which acts as an antenna, will be attached to a bond pad formed on the chip using methods known to those skilled in the art. The wire will then be stabilized when the chip is encased in the protective shell, so that the antenna extends at some angle to the chip.

Also, as an alternative to signal transmission via RF, the antifuse or other semiconductor memory and supporting circuitry can receive the addressing commands and device power by optical transmission. In this embodiment, the RF antenna 110 would be replaced by a photocell that generates sufficient write voltage to exceed the threshold voltage. For the addressing commands, the RF transmitter 80 is replaced by a light source, and the commands may be transmitted digitally by pulsing the optical transmitter, which can be a laser, flash lamp or other high intensity light source. It is noted that the light intensity must be sufficient to generate adequate voltage, either singly or in conjunction with a second power generating device, in the photocell to write into memory, but not so high that it damages the metal interconnect on the chip. With digital data transmission analog-to-digital and digital-to-analog conversion circuitry can be eliminated.

3. Other memory devices and encoded devices

In addition to antifuse memory devices, other types of electrically-programmable read-only memories, preferably non-volatile memories, which are known in the art, may be used [see, e.g., U.S. Patent No. 5,335,219]. Chips, such as those sold by Actel, Mosaic, Lattice Semiconductor, AVID, Anicare, Destron, Rayethon, Altera, ICT, Xilinix, Intel and Signetics [see, e.g., U.S. Patent Nos. 4,652,528, 5,044,623, 5,099,226, 5,218,343, 5,323,704, 4,333,072, 4,321,069, 4,318,658, 5,121,748, 5,214,409, 5,235,326, 5,257,011 and 5,266,926] may be used herein. Preprogrammed remotely addressable identification tags, such as those used for tracking objects or animals [see, e.g., U.S. Patent Nos. 5,257,011, 5,235,326, 5,226,926, 5,214,409, 4,333,072, available from AVID, Norco,

CA; see, also U.S. Patent No. 5,218,189, 5,416,486, 4,952,928, 5,359,250] and remotely writable versions thereof are also contemplated for use herein. Preprogrammed tags may be used in embodiments, such as those in which tracking of linked molecules is desired. Devices sold by XCI [San Jose, CA] that operate in the lower frequency [~900 mhz] range are also preferred herein.

4. Pre-coded memory devices

Alternatively, the matrices or strips attached thereto may be encoded with a pre-programmed identifying bar code, such as an optical bar code that will be encoded on the matrix and read by laser. Such pre-coded devices may be used in embodiments in which parameters, such as location in an automated synthesizer, are monitored. The identity of a product or reactant determined by its location or path, which is monitored by reading the chip in each device and storing such information in a remote computer. Read/write tags such as the IPTT-100 [BioMedic Data Systems, Inc., Maywood, NJ; see, also U.S. Patent Nos. 5,422,636, 5,420,579, 5,262,772, 5,252,962, 5,250,962, and U.S. application Serial No. 08/322,644] are also contemplated for use herein.

Among the particularly preferred devices are the chips [particularly, the IPTT-100, Bio Medic Data Systems, Inc., Maywood, NJ; see, also U.S. Patent Nos. 5,422,636, 5,420,579, 5,262,772, 5,252,962 and 5,250,962 and U.S. application Serial No. 08/322,644,] that can be remotely encoded and remotely read. These devices, such as the IPTT-100 transponders that are about 8 mm long, include a recording device, an EEPROM, a passive transponder for receiving an input signal and transmitting an output signal in response. In some embodiments here, the devices are modified for use herein by altering the geometry. They are folded in half and the antenna wrapped around the resulting folded structure. This permits convenient insertion into the microvessels and formation of other combinations.

These devices include a power antenna means [see, e.g., U.S. Patent No. 5,250,944 and U.S. Patent No. 5,420,579] for receiving the input

signal, frequency generator and modulator means for receiving the input signal the receive antenna means and for generating the output signal. The output signal has a frequency different from the input frequency, outputs the output signal in response the input signal. The input signal having a first frequency, the output signal has a second frequency that is a multiple of the first frequency, and is greater that the first frequency. It also includes a transmitting antenna means for receiving the output signal from the frequency generator and modulator means and that transmit the output signal. Data are stored within the transponder within a reprogrammable memory circuit that is programmed by the user [see, e.g., U.S. Patent No. 5,422,636 and EP 0 526 173 A3]. A transponder scanner for scanning and programming the transponder is also available [Bio Medic Data Systems Inc. DAS-5001 CONSOLE™ System, e.g., U.S. Patent No. 5,252,962 and U.S. Patent No. 5,262,772].

5. Other memories

Another such device is a 4 mm chip with an onboard antenna and an EEPROM [Dimensional Technology International, Germany]. This device can also be written to and read from remotely.

Also, ID tags available from IDTAG™ Inc, particularly the IDT150 read/write transponder [ITDAG™ Ltd. Bracknell, Berks RG12 3XQ, UK], and IDT101, discussed above, are also preferred herein.

6. Monolithic semiconductor tags

Additionally, smaller [about 2 mm x 2 mm x 0.1 mm or less] monolithic devices are of interest herein. These are particularly suitable for use with the MICROTUBE® microreactors and similar devices because they can be readily encased in these devices. For example, in a particular embodiment of an electromagnetically programmable tag, such as, for examplification purposes an RF or microwave tag, a single chip tag is formed entirely on a single substrate. More specifically, referring to FIGURE. 43, a monolithic tag, such as an RF or microwave tag, is shown and generally designated 4700. This monolithic tag is sized such that the

substrate has following overall external dimensions: width 4710 of 2mm wide, length 4708 of 2mm, and a height 4714 of 0.1mm. As a result of this miniaturization of the tag, a variety of shapes and sizes of the tags may be created. More specifically, United States Patent No. 4,857,893 issued to Carroll in 1989, entitled "Single Chip Transponder Device" describes a single substrate (monolithic) RF transponder. Modification by suitable selection of dimensions of the device to render it suitable for operation in the microwave range is also contemplated herein.

In FIGURE 43, the tag 4700 is shown having a substrate 4702 is formed with an antenna 4714. This antenna is preferably formed on the substrate using a metalization process wherein a metal is placed on a pattern on the top surface of the substrate to create a particular antenna. As shown, the antenna is substantially square, tracing out a coiled antenna beginning at pad 4712, and ending at conductor 4706 which attaches back to the circuitry 4704. It should be appreciated that while the antenna is shown to be square, any other shapes could be used. In particular, a circular antenna could be formed just as easily on the surface of the substrate. It should be appreciated, however, that the functionality of the antenna are likely very similar between a square antenna and a circular antenna. In addition to the antenna as shown, there may be a second antenna on the back side of the substrate (not shown) which could be used to increase the number of windings or, as an alternative, be tuned for a different frequency range than the antenna patterned on the upper surface of the substrate 4702.

Referring to FIGURE 44, the tag is shown in plan view and has circuitry 4704 which includes specific logic and control electronics generally denoted 4706. The pattern of the antenna 4714 is easily appreciated from this view. Moreover, the circular equivalent can be easily envisioned on the substrate 4702 to spiral around the circuitry 4704 to create a similarly sized antenna.

The specific electronic circuitry that is contained in circuitry 4704 is well described in both the United States Patent No. 4,857,893, and United States Patent No. 5,345,231, discussed above, and both fully incorporated herein by reference. United States Patent No. 5,345,231, entitled "Contactless Inductive Data-Transmission System" discloses circuitry capable of communicating identification information across a wireless communication system which employs an inductive coupling. Specifically, with reference to Fig. 2 of that reference, this inductive coupling provides the power to run the tag electronics, as well as provides the communication channel with which the identification information travels. The circuitry includes a rectifier attached to the antenna to receive an electromagnetically coupled signal, and to create its own power from the signal. In addition to the rectifier, a clock extractor and demodulator also receive the antenna's signal. The clock extractor recreates a communication clock, and the demodulator decodes the signal received from the antenna using that clocking information. This information is provided to a control unit which either programs or downloads the contents of a memory bank. In the particular tag discussed herein, the memory bank can include a single data bit, or may be easily expanded as is generally known in the art to a variety of memory sizes, up to several kilobytes. Once the memory has been accessed, the control unit can communicate with the base transmitter/receiver by sending data to the modulator which is also electrically connected to the antenna. In that manner, the antenna can be used to either receive a signal from the transmitter, or to transmit a signal to the receiver.

The antenna for use with these particular electronics is tuned for a resonant frequency of approximately 125 kHz. It is to be appreciated, as discussed above, that the antenna can easily be tuned to receive a variety of frequencies, particularly microwave frequencies. It should be noted, however, that depending on the frequencies to be received, the antenna shape and size could be altered. In fact, it should be appreciated that a

single antenna which is capable of receiving a number of frequencies could be created by having electrical leads which attached to a number of points along the length of the antenna. As a result, a single antenna could be used to receive a number of frequencies, where the frequency to be received could be set by an initial communication with the tag. Because of such frequency specification, it should be appreciated that the a number of tags could be addressed simultaneously in a "batch" read or write process.

A batch read/write process permits a single transmitter station to address more than one tag at the same time. This is particularly useful when desiring to program a number of tags with the same or similar information. Thus, by batch writing the information to a number of tags, the programming process is shortened and simplified, while at the same time, minimizing the opportunity for error.

In addition to the ease and accuracy of batch accessing the tags, such a feature is particularly useful when faced with a large number of tags to identify. More particularly, the ability to batch access the tags permits the transmitter/receiver to identify any number of tags within an area simply by one access process instead of having to access each tag individually. It should be appreciated that in addition to simply accessing all tags at one time, there could be a variety of signal types which could narrow the field of access. One manner of restricting the access to tags, such as an RF tag, could be to make any interrogation either code or frequency discriminating. Such discrimination would occur, for example, by selecting only the particular RF tags within a frequency range. On the other hand, the specific address code of a number of RF tags could be programmed such that by identifying, for example, the first four of an eight bit address scheme, only a portion of the RF tags identifiable with those eight addressing bits could be addressed.

In United States Patent No. 4,857,893 for a "Single Chip Transponder Device" the circuitry for communication with a transmitting and receiving base is shown generally in Fig. 2 of that reference and is

incorporated herein by reference. An antenna receives a carrier signal which is provided to a rectifier and demodulator, as well as a timing decoder. The rectifier captures a portion of the carrier signal to derive dc power to drive the RF tag itself. The timing decoder uses the remaining portion of the antenna signal to derive the timing signals necessary to control the data storage and generation of the RF tag. Once accessed, the data generator creates a data signal which is modulated and supplied to the antenna for retransmit back to the transmitter/receiver unit.

In addition to allowing the simple tracking of various containers, it is possible to place a plurality of tags, such as different RF tags, within a container that is already tagged. More specifically, it is possible to place a tag in a container that is already identified with one tag. This combining of multiple tags would permit a greater level of tracking of the container. For instance, a beaker could be formed with a RF tag integral to its structure. Once identified, the beaker may be filled with a variety of materials, each having its own identification number. Thus, when solution B is added to beaker A,. an RF tag indicating the material B can simply be dropped into the beaker A. Likewise, when solution C is added to beaker A, an RF tag indicating the material C can be dropped in the beaker. As a result of this marking method, it would be possible to verify the exact contents of a container. Specifically, by reading the various RF tags within the beaker A (solution B and solution C) the entire contents of the container would be identified.

Alternatively, in addition to, or instead of, simply identifying the contents of a container, it would also be possible to track the whereabouts of a container by adding identifying RF tags at various locations in its path. For instance, a beaker could be marked with an identifying RF tag A, and an identifying RF tag B could be added when a particular process is performed on the contents of the beaker A. Similarly, an identifying RF tag C could be added to the beaker at the next process step. Thus, the RF tags in the

beaker would indicate the exact historical location of the container A simply by decoding the contents of the RF tags contained therein.

In addition, the tags could be used to provide a combination of the above described contents and location based information. This combination would provide a means to analyze a "chain of possession" for various contents of a container. In other words, the contents of a beaker could be determined by identifying the RF tags. This information would provide a history of the contents of the container, as well as location of the processes which were performed on the container.

7. Rhodopsins

Another memory means that is suitable for use in the matrix with memory combinations are optical memories that employ rhodopsins, particularly bacteriorhodopsin [BR], or other photochromic substances that change between two light absorbing states in response to light of each of two wavelengths [see, e.g., U.S. Patent No. 5,346,789, 5,253,198 and 5,228,001; see, also Birge (1990) Ann. Rev. Phys. Chem 41:683-733]. These substances, particularly BR, exhibit useful photochromic and optoelectrical properties. BR, for example, has extremely large optical nonlinearities, and is capable of producing photoinduced electrical signals whose polarity depends on the prior exposure of the material to light of various wavelengths as well as on the wavelength of the light used to induce the signal. There properties are useful for information storage and computation. Numerous applications of this material have been designed, including its use as an ultrafast photosignal detector, its use for dynamic holographic recording, and its use for data storage, which is of interest herein.

The rhodopsins include the visual rhodopsins, which are responsible for the conversion of light into nerve impulses in the image resolving eyes of mollusks, anthropods, and vertebrates, and also bacteriorhodopsin [BR]. These proteins also include a class of proteins that serve photosynthetic and phototactic functions. The best known BR is the only protein found in

nature in a crystalline membrane, called the "purple membrane" of Halobacterium Halobium. This membrane converts light into energy via photon-activated transmembrane proton pumping. Upon the absorption of light, the BR molecule undergoes several structural transformations in a well-defined photocycle in which energy is stored in a proton gradient formed upon absorption of light energy. This proton gradient is subsequently utilized to synthesize energy-rich ATP.

The structural changes that occur in the process of light-induced proton pumping of BR are reflected in alterations of the absorption spectra of the molecule. These changes are cyclic, and under usual physiological conditions bring the molecule back to its initial BR state after the absorption of light in about 10 milliseconds. In less than a picosecond after BR absorbs a photon, the BR produces an intermediate, known as the "J" state, which has a red-shifted absorption maximum. This is the only light-driven event in the photocycle; the rest of the steps are thermally driven processes that occur naturally. The first form, or state, following the photon-induced step is called "K", which represents the first form of light-activated BR that can be stabilized by reducing the temperature to 90 ° K. This form occurs about 3 picoseconds after the J intermediate at room temperature. Two microseconds later there occurs an "L" intermediate state which is, in turn, followed in 50 microseconds by an "M" intermediate state.

There are two important properties associated with all of the intermediate states of this material. The first is their ability to be photochemically converted back to the basic BR state. Under conditions where a particular intermediate is made stable, illumination with light at a wavelength corresponding to the absorption of the intermediate state in question results in regeneration of the BR state. In addition, the BR state and intermediates exhibit large two-photon absorption processes which can be used to induce interconversions among different states.

The second important property is light-induced vectorial charge transport within the molecule. In an oriented BR film, such a charge transport can be

detected as an electric signal. The electrical polarity of the signal depends on the physical orientation of molecules within the material as well as on the photochemical reaction induced. The latter effect is due to the dependence of charge transport direction on which intermediates [including the BR state] are involved in the photochemical reaction of interest. For example, the polarity of an electrical signal associated with one BR photochemical reaction is opposite to that associated with a second BR photochemical reaction. The latter reaction can be induced by light with a wavelength around 412 nm and is completed in 200 ns.

In addition to the large quantum yields and distinct absorptions of BR and M, the BR molecule [and purple membrane] has several intrinsic properties of importance in optics. First, this molecule exhibits a large two-photon absorption cross section. Second, the crystalline nature and adaptation to high salt environments makes the purple membrane very resistant to degeneration by environmental perturbations and thus, unlike other biological materials, it does not require special storage. Dry films of purple membrane have been stored for several years without degradation. Furthermore, the molecule is very resistant to photochemical degradation.

Thus, numerous optical devices, including recording devices have been designed that use BR or other rhodopsin as the recording medium [see, e.g., U.S. Patent No. 5,346,789, 5,253,198 and 5,228,001; see, also Birge (1990) Ann. Rev. Phys. Chem 41:683-733]. Such recording devices may be employed in the methods and combinations provided herein.

D. Event-detecting embodiment and combinations with sensors

Another embodiment of the combinations herein uses a recording device that can detect the occurrence of a reaction or event or the status of any external parameter, such as pH or temperature, and record a such occurrence or parameter in the memory. Any of the above devices may be modified to permit such detection. For example, the chip with the antifuse memory array with decoder, rectifier components and RF antenna, can be modified by addition of a photodetector and accompanying amplifier

components as shown in Figure 9. The photodetector will be selected so that it is sensitive to the frequencies of expected photoemissions from reactions of interest. To maintain the chip's passive operation, the photodetector circuitry may use voltage supplied by the same RF signal that is used to write other data to memory, so that no detection of photoemission will occur unless RF or other power is applied to provide bias and drain voltage. If an active device is used, the power supplied by the battery can provide operational voltage to the photodetector circuitry, independent of any transmitted signal. The voltage supplied by the photodetector can be used in a number of different ways. For example:

1) The threshold voltage for writing to memory will exceed the voltage supplied by the RF signal, which will still contain the address information. In order to write, additional voltage must be provided by the photodetector so that the sum of the voltages exceeds the threshold. ($V_{RF} < V_T < V_{RF} + V_{PD}$). This permits the RF supplied voltage to go to the correct address, however, no writing will occur unless a photoemission has been detected by the detector. Therefore, there will be no record of exposure to a particular process step unless a sufficient reaction has occurred to generate the required photoemission. Since the address signal can still get to the memory array without the extra voltage, reading of recorded data can be achieved without any special circuitry. If the memory device is an active device, a similar mechanism can be used in which only the sum of the voltages is sufficient to record an occurrence.

2) The threshold voltage for writing to memory will be provided by the RF signal alone, and the RF signal will include address information. ($V_T < V_{RF}$). Unless voltage from the photodetector is supplied to a "gating" transistor, access to the memory array is prevented so that no writing occurs unless a photoemission is detected. (This embodiment is illustrated.) This will require a special provision for opening the gate during read operations to permit access to the memory array. Since the gating

transistor will conduct a signal only in the event of photoemission, this embodiment will work equally well with passive and active memory devices.

3) The RF signal provides sufficient voltage to exceed the threshold voltage. ($V_T < V_{RF}$). Voltage from the photodetector is used to create a write potential difference at an additional address location which is carried in the RF signal. For example, if the RF signal is addressing column 3, row 3, column 32 could be connected only to the photodetector circuit's output so that, when a photoemission occurs, the write signal will create antifuses (or in the case of EEPROM, standard fuses) at addresses 3,3 and 32,3. If no photoemission occurs, only address 3,3 will have an antifuse formed, providing a record of exposure of the matrix to a particular process step even without the occurrence of a detectable reaction. Special provisions, such as software within the host computer in combination with mask-programmed interconnections within the decode circuitry of the memory device, must be made to assure that more than one column in a single row of the array is polled during read operations so that both memory locations are read.

In addition to the above-described methods for recording the occurrence of photo-emitting reactions, the photodetector, while still integrated on the same substrate with the basic memory matrix for recording transmitted signals, can be connected to its own independent memory matrix. In this embodiment, the photodetector's memory matrix can be connected to separate transceiver circuitry with an antenna tuned to a different frequency from that of the basic memory. During the read operation, the memory device will be exposed to two different radio frequency signals, one for the basic memory, the other for the photodetection circuit memory. If only the photoemission information is required, only the corresponding frequency signal need be provided during the read operation.

Depending on the type of energy release that occurs during a reaction, other types of sensors may be used in addition to photodetectors

or in place thereof. In addition changes in ion concentration may also be detected. Many such sensors will be capable of generating an electrical signal that can be used as described above for the photodetectors. These sensing devices may also be incorporated onto the substrate and electrically connected to the memory device, providing data points within the device's memory under the appropriate write conditions. For example, temperature sensing elements can be made from semiconductor liquid crystal and fluorescent crystals, and addition to conventional thermocouples created by placing two different metals in contact at the detection point. It is also possible to include radiation, pH and pCO_2 sensors in a similar manner, using materials that respond to the detected variables by generating a voltage potential that can be conducted to the memory device and recorded.

The reaction-detecting embodiment may be advantageously used in assays, such as the SPA, HTRF, FET, FRET and FP assays described below. In these assays, reaction, such as receptor binding, produces a detectable signal, such as light, in the matrix. If a matrix with memory with a photodetection circuit is used, occurrence of the binding reaction will be recorded in memory.

E. Reading and writing to memory

1. Embodiments using a proximate memory, such as a non-volatile memory device

The operation of programming the memory to record the process steps to which the linked or adjacent matrix particle or support and linked or proximate molecule or biological particle is exposed involves placing the memory device reasonably close [a distance on the order of about 1 inch [25.4 mm]] is presently contemplated, but longer distances should be possible and shorter distances are also contemplated [suitable distances can be determined empirically] to RF (or microwave) transmitter 80. The distance is a function the transmitter and selected frequency. The following discussion is made with reference to RF, but is applicable to other selected frequencies, including microwave and radar.

The transmitter 80 emits a carrier wave modulated by a signal generated by host computer 122 using conventional RF technology. The carrier wave provides the power to the generate the programming voltage and the operating voltage for the various devices via the rectifier, while the modulation signal provides the address instructions.

As stated previously, since the memory only has to be "tagged" to record the exposure of the proximate or linked molecule or biological particle to a given process, the address signal only has to carry information to turn on a single memory location, while the host computer 122 stores into memory 120 the information linking the process information with the single memory location that was "tagged" to record exposure to the process step. Referring to Figure 1, in which chemical building blocks A, C, and E are added to a molecule linked to a matrix with memory, and to Figure 6, an illustrative example of how information is written into the memory of the combination is provided in Table 1.

TABLE 1

PROCESS STEP	X-REGISTER ADDRESS	Y-REGISTER ADDRESS
A	1	8
C	2	4
E	3	2

For the step in which A is added, the address signal would increment the x-register 124 one location and increment the y-register 126 eight locations, and then apply the programming voltage. The activation of this switch is indicated by an "A" at the selected address, although the actual value stored will be a binary "1", indicating ON. [As described, for example, in U.S. Patent No. 4,424,579; the manner in which the programming voltage is applied depends on whether the decoders have depletion or enhancement transistors.] The host computer 122 would write into its memory 120 that for process A, the x-,y- address is 1,8. Upon removal of the RF signal after recording process A, the voltage is removed and the registers would reset to 0. For the step in which C is added, the address signal would increment the x-register 124 two locations and the y-register

126 four locations, then apply the programming voltage, as indicated by the letter "C". The host computer 120 would similarly record in memory that an indication of exposure to process C would be found at x-,y- address 2,4. Again, upon removal of the RF signal, the registers reset to 0 so that when the matrix memory is again exposed to RF following addition of block E, the registers increment 3 and 2 locations, respectively, and the programming voltage is applied to turn on the switch, indicated by "E". Desirably all processing steps are automated.

After processing is completed, to read the information that has been recorded in the memory of the data storage unit, the host computer 122 will inquire into the identity of the molecule by generating a command signal to the registers to select the appropriate address locations to determine whether the switch is on or off. If the switch is on, i.e., a voltage drop occurs at that point, the computer will create a record that the molecule received a particular process step. Alternatively, the host computer can generate an inquiry signal to sequentially look at all memory locations to determine which switches have been turned on, recording all locations at which voltage drops occurred. The computer will then compare the "on" locations to the process steps stored in its memory to identify the steps through which the subject molecule was processed.

If desired, individual compounds can be identified by reserving certain memory locations for identification only, for example, the first two rows of the x-register. In this case, combinations of matrix with memories with linked or proximate molecules will be passed separately through the RF signal while the x-register is incremented to turn on switches at address locations 0,0, 1,0, 2,0, etc. With individual identification, the host computer 122 can first generate a signal to query a matrix memory to determine its identity, then write the information with regard to the process performed, saving the process and particle information in the host computer memory 120.

The tagging of molecules, which are exposed to a particular process is performed in the process vessel containing all of the particles. The presence, however, of a large number of particles may result in interference or result in an inability to generate a sufficiently high voltage for programming all of the particles simultaneously. This might be remedied by providing an exposure of prolonged duration, e.g., several minutes, while stirring the vessel contents to provide the greatest opportunity for all particles to receive exposure to the RF signal. On the other hand, since each particle will need to be read individually, a mechanism for separating the combinations may be used in both write and read operations and subsequent operations. Also, in instances in which each matrix memory will have a different molecule attached, each memory must be addressed separately.

2. Embodiments using OMDs

When precoded OMDs are used, each OMD (or group thereof) has a unique identifier is optically scanned and entered into a remote memory. Thereafter, after each synthesis, processing or assaying step, information regarding such for each device identified by its encoded symbology is entered into a remote memory. Upon completion of the synthesis, processing, assay or other protocol, each device can be scanned and identified. Reference to the information stored in the remote memory provides information regarding the linked molecules or biological particles or the assay or other information. When read/write OMDs are used, identifying symbology is encoded on the device and the decrypting information is stored in a remote memory.

D. The combinations and preparation thereof

Combinations of a miniature recording device that contains or is a data storage unit linked to or in proximity with matrices or supports used in chemical and biotechnical applications, such as combinatorial chemistry, peptide synthesis, nucleic acid synthesis, nucleic acid amplification methods, organic template chemistry, nucleic acid sequencing, screening for

drugs, particularly high throughput screening, phage display screening, cell sorting, drug delivery, tracking of biological particles and other such methods, are provided. These combinations of matrix material with data storage unit [or recording device including the unit] are herein referred to as matrices with memories. These combinations have a multiplicity of applications, including combinatorial chemistry, isolation and purification of target macromolecules, capture and detection of macromolecules for analytical purposes, high throughput screening protocols, selective removal of contaminants, enzymatic catalysis, drug delivery, chemical modification, scintillation proximity assays, FET, FRET and HTRF assays, immunoassays, receptor binding assays, drug screening assays, information collection and management and other uses. These combinations are particularly advantageous for use in multianalyte analyses. These combinations may also be advantageously used in assays in which a electromagnetic signal is generated by the reactants or products in the assay. These combinations may be used in conjunction with or may include a sensor element, such as an element that measures a solution parameter, such as pH. Change in such parameter, which is recorded in the memory will indicate a reaction event of interest, such as induction of activity of a receptor or ion channel, has occurred. The combination of matrix with memory is also advantageously used in multiplex protocols, such as those in which a molecule is synthesized on the matrix, its identity recorded in the matrix, the resulting combination is used in an assay or in a hybridization reaction. Occurrence of the reaction can be detected externally, such as in a scintillation counter, or can be detected by a sensor that writes to the memory in the matrix. Thus, combinations of matrix materials, memories, and linked or proximate molecules and biological materials and assays using such combinations are provided.

The combinations contain (i) a miniature recording device that contains one or more programmable data storage devices [memories] that can be remotely read and in preferred embodiments also remotely

programmed; and (ii) a matrix as described above, such as a particulate support used in chemical syntheses. The remote programming and reading is preferably effected using electromagnetic radiation, particularly radio frequency, microwave, X-rays or radar. Depending upon the application the combinations will include additional elements, such as scintillants, photodetectors, pH sensors and/or other sensors, and other such elements.

1. Preparation of matrix-memory combinations

In preferred embodiments, the recording device is cast in a selected matrix material during manufacture. Alternatively, the devices can be physically inserted into the matrix material, the deformable gel-like materials, or can be placed on the matrix material and attached by a connector, such as a plastic or wax or other such material. Alternatively, the device or device(s) may be included in an inert container in proximity to or in contact with matrix material.

2. Non-linked matrix-memory combinations

The recording device with memory can be placed onto the inner or outer surface of a vessel, such as a microtiter plate or vial or tube in which the reaction steps are conducted, fractions collected or samples stored. Alternatively, the device can be incorporated into the vessel material, such into the a wall of each microtiter well or vial or tube in which the reaction is conducted. As long as the molecules or biological particles remain associated with the well, tube or vial, their identity can be tracked. The memory will be a programmable electronic memory or a bar code. These memories can also be associated with reagent containers.

A bar code reader, transponder reader or other such device can be used to enter the desired inforfmation building block name by reading the reagent container. Such information will be entered in to matrix memory or remote computer memory. Software for doing so can be integrated into the systems used, such as the bar code reader described herein or the transponders and encoders described herein.

Also of interest herein are the multiwell "chips" [such as those available from Orchid Biocomputer, Inc. Princeton, NJ, see, e.g.,U.S. Patent Nos. 5,047,371, 4,952,531, 5,043,222, 5,277,724, 5,256,469 and Prabhu et al. (1992) Proc. SPIE-Int. Soc. Opt. Eng. 1847 NUMBER: Proceedings of the 1992 International Symposium on Microelectronics, pp.601-6], that are silicone based chips that contain 10,000 microscopic wells connected by hair-thin glass tubes to tiny reservoirs containing reagents for synthesis of compounds in each well. Each well can be marked with a code and the code associated with the identity of the synthesized compound in each well. Ultimately, a readable or read/write memory may be incorporated into each well, thus permitting rapid and ready indentification of the contents of each well.

In a particularly preferred embodiment, one or more recording devices with memory and matrix particles are sealed in a porous non-reactive material, such as polypropylene or TEFLON net, with a pore size smaller than the particle size of the matrix and the device. Typically one device per about 1 to 50 mg, preferably 5 to 30, more preferably 5 to 20 mg of matrix material, or in some embodiments up to gram, generally 50 to 250 mg, preferably 150 mg to about 200 mg, and one device is sealed in a porous vessel a microvessel [MICROKAN™]. The amount of matrix material is a function of the size of the device and the application in which the resulting matrix with memory is used, and, if necessary can be empirically determined. Generally, smaller sizes are desired, and the amount of material will depend upon the size of the selected recording device.

The resulting microvessels are then encoded, reactions, such as synthetic reactions, performed, and read, and if desired used in desired assays or other methods.

3. Preparation of matrix-memory-molecule or biological particle combinations

In certain embodiments, combinations of matrices with memories and biological particle combinations are prepared. For example, libraries [e.g.,

bacteria or bacteriophage, or other virus particles or other particles that contain genetic coding information or other information] can be prepared on the matrices with memories, and stored as such for future use or antibodies can be linked to the matrices with memories and stored for future use.

4. Combinations for use in proximity assays

In other embodiments the memory or recording device is coated or encapsulated in a medium, such as a gel, that contains one or more fluophors or one or more scintillants, such as 2,5-diphenyloxazole [PPO] and/or 1,4-bis-[5-phenyl-(oxazolyl)]benzene [POPOP] or FlexiScint [a gel with scintillant available from Packard, Meriden, CT] or yttrium silicates. Any fluophore or scintillant or scintillation cocktail known to those of skill in the art may be used. The gel coated or encased device is then coated with a matrix suitable, such as glass or polystyrene, for the intended application or application(s). The resulting device is particularly suitable for use as a matrix for synthesis of libraries and subsequent use thereof in scintillation proximity assays.

Similar combinations in non-radioactive energy transfer proximity assays, such as HTRF, FP, FET and FRET assays, which are described below. These luminescence assays are based on energy transfer between a donor luminescent label, such as a rare earth metal cryptate [e.g., Eu trisbipyridine diamine (EuTBP) or Tb tribipyridine diamine (TbTBP)] and an acceptor luminescent label, such as, when the donor is EuTBP, allopycocyanin (APC), allophycocyanin B, phycocyanin C or phycocyanin R, and when the donor is TbTBP, a rhodamine, thiomine, phycocyanin R, phycoerythrocyanin, phycoerythrin C, phycoerythrin B or phycoerythrin R. Instead of including a scintillant in the combination, a suitable fluorescent material, such as allopycocyanin (APC), allophycocyanin B, phycocyanin C, phycocyanin R; rhodamine, thiomine, phycocyanin R, phycoerythrocyanin, phycoerythrin C, phycoerythrin B or phycoerythrin R is included. Alternatively, a fluorescent material, such a europium cryptate is incorporated in the combination.

5. 2-D Bar codes, other symbologies and application thereof

Any application and combination described herein in which a recording device in proximity with a matrix may include a code or symbology in place of or in addition to the recording device. The information associated with the code is stored in a remote recording device, such as a computer. Thus, by electro-optically scanning the symbol on the combination and generating a corresponding signal, it is possible in an associated computer whose memory has digitally stored therein the full range of codes, to compare the signal derived from the scanned symbol with the stored information. When a match is found, the identity of the item and associated information, such as the identity of the linked molecule or biological particle or the synthetic steps or assay protocol, can be retrieved.

The symbology can be engraved on any matrix used as a solid support for chemical syntheses, reactions, assays and other uses set forth herein, for identification and tracking of the linked or proximate biological particles and molecules. Particularly preferred is the two-dimensional bar code and system used therewith for reading and writing the codes on matrix materials.

6. Other variations and embodiments

The combination of matrix particle with memory may be further linked, such as by welding using a laser or heat, to an inert carrier or other support, such as a TEFLON strip. This strip, which can be of any convenient size, such as 1 to 10 mm by about 10 to 100 μM will render the combination easy to use and manipulate. For example, these memories with strips can be introduced into 10 cm culture dishes and used in assays, such as immunoassays, or they can be used to introduce bacteria or phage into cultures and used in selection assays. The strip may be encoded or impregnated with a bar code to further provide identifying information.

Microplates containing a recording device in one or a plurality of wells are provided. The plates may further contain embedded scintillant or a coating of scintillant [such as FlashPlate™, available from DuPont NEN®, and plates available from Packard, Meriden, CT] FLASHPLATE™ is a 96 well microplate that is precoated with plastic scintillant for detection of β-emitting isotopes, such as ^{125}I, ^{3}H, ^{35}S, ^{14}C and ^{33}P. A molecule is immobilized or synthesized in each well of the plate, each memory is programmed with the identify of each molecule in each well. The immobilized molecule on the surface of the well captures a radiolabeled ligand in solution results in detection of the bound radioactivity. These plates can be used for a variety of radioimmmunoassays [RIAs], radioreceptor assays [RRAs], nucleic acid/protein binding assays, enzymatic assays and cell-based assays, in which cells are grown on the plates.

Another embodiment is depicted in FIGURE 19. The reactive sites, such as amines, on a support matrix [1 in the FIGURE] in combination with a memory [a MICROKAN™, a MICROTUBE™, a MACROBEAD™, a MICROCUBE™ or other matrix with memory combination] are differentiated by reacting them with a selected reation of Fmoc-glycine and Boc-glycine, thereby producing a differentiated support [2]. The Boc groups gropus on 2 are then deprotected with a suitable agent such as TFA, to produce 3. The resulting fee amine groups are coupled with a fluophore [or mixture A and B, to produce a fluorescent support 4, which can be used in subsequent syntheses or for linkage of desired molecules or biological particles, and then used in fluorescence assays and SPAs.

E. The recording and reading and systems

Systems for recording and reading information are provided. The systems include a host computer or decoder/encoder instrument, a transmitter, a receiver and the data storage device. The systems also can include a funnel-like device or the like for use in separating and/or tagging single memory devices. In practice, an EM signal, preferably a radio frequency signal is transmitted to the data storage device. The antenna or other

receiver means in the device detects the signal and transmits it to the memory, whereby the data are written to the memory and stored in a memory location.

Mixtures of the matrix with memory-linked molecules or biological particles may be exposed to the EM signal, or each matrix with memory [either before, after or during linkage of the biological particles or molecules] may be individually exposed, using a device, such as that depicted herein, to the EM signal. Each matrix with memory, as discussed below, will be linked to a plurality of molecules or biological particles, which may be identical or substantially identical or a mixture of molecules or biological particles depending, upon the application and protocol in which the matrix with memory and linked [or proximate] molecules or biological particles is used. The memory can be programmed with data regarding such parameters.

The location of the data, which when read and transmitted to the host computer or decoder/encoder instrument, corresponds to identifying information about linked or proximate molecules or biological particles. The host computer or decoder/encoder instrument can either identify the location of the data for interpretation by a human or another computer or the host computer or the decoder/encoder can be programmed with a key to interpret or decode the data and thereby identify the linked molecule or biological particle.

As discussed above, the presently preferred system for use is the IPTT-100 transponder and DAS-5001 CONSOLE™ [Bio Medic Data Systems, Inc., Maywood, NJ; see, e.g., U.S. Patent Nos. 5,422,636, 5,420,579, 5,262,772, 5,252,962 and 5,250,962, 5,252,962 and 5,262,772].

These systems may be automated or may be manual. The manual system is described herein; an automated systems are described in copending, commonly owned U.S. application Serial No. 08/826,253.

Manual sytem

The presently preferred manual system includes a transponder, particularly the BMDS transponder described below or an IDTAG™ transponder, described above, and uses the corresponding reading and writing device, which has been reconfigured and repackaged, such as in FIGURE 17, described in the EXAMPLES An example of the operation of the system of FIGURE 17 is illustrated in FIGURE 18 and described in EXAMPLE 4. Briefly, the user manually places a microvessel 180 within the recessed area 176 so that the interrogation signal 185 provides a response to the controllers indicating the presence on the microvessel, and information is read from or written to the transponder..

This will include microvessels, such as MICROKANS™ or MICROTUBES™, read/writer hardware [such as that available from BMDS or IDTAG™] connected to a PC and software running on the PC that performs a user interface and system control function. The software is designed to facilitate the a number of aspects of synthetic combinatorial chemistry libraries, including: organization, planning and design, synthesis compound formula determination, molecular weight computation, reporting of plans, status and results.

In particular, for each chemical library, the software creates a data base file. This file contains all of the information pertinent to the library, including chemical building blocks to be used, the design of the library in terms of steps and splits, and what synthesis has been performed. This file oriented approach allows many different chemical library projects to be conducted simultaneously. The software allows the user to specify what chemical building blocks are to be used and their molecular weights. The user specifies the number of steps, the number of "splits" at each step, and what chemical building blocks are to be used at each split. The user may also enter the name of the pharmacophore and its molecular weight. Additionally, the user may specify graphical chemical diagrams for the building blocks and the pharmacophore. This information is useful in

displaying resulting compounds. The software records all of the above "design" information. It computes and displays the size of the library. It may also predict the range of molecular weights of the resulting compounds.

For example, the user specifies that there will be eight chemical building blocks. Their names are entered, and the user enters a unique letter codes for each: A, B, C, D, E, F, G and H. The user specifies that there will be three steps. Step one will have four splits, appending the A, B, C and D building blocks. Step two will also have four splits, adding the B, D, E and H building blocks. Step three will have six splits, adding the B, C, D, E, F and G building blocks. The software computes that the library will contain 96 (4 x 6 x 5 = 96) unique compounds. With the planning and design completed, the software helps the user perform the synthesis steps. This is done in concert with the reader/writer hardware [transceiver or a scanner, such as the BMDS - DAS 5003] or a similar device available from IDTAG Ltd [Bracknell, Berks RG12 3XQ, UK] and devices, such as the MICROKAN™ or MICROTUBE™ microvessel with memory devices. Before the synthesis begins, the microvessels are filled with polymer resin. The microvessel devices are, one at a time placed upon the scanner. The device and software reads the contents of the data encoded in the recording device, transponder, such as the BMDS tag or the IDTAG™ tag, contained in each microvessl. The software, chooses which building block shall be added to the compound contained in each microvessel. It directs the transceiver to write encoded data to the transponder, indicating which building block this is. The software displays a message which directs the user to place the microvessel in the appropriate reaction vessel so that the chosen building block will be added. This process is repeated a plurality of times with each microvessel and for each synthetic step the planned steps of the library.

The software then uses the scanner to read a tag and receive its encoded information. Using the user-entered compound names stored in the library's data base, the software translates the encoded information into the

names of the chemical building blocks. The software can also display compounds graphically, using the graphical information specified by the user. The software calculates the molecular weight of compounds from the data provided for the pharmacophore and building blocks. The software facilitates the recording of progress through the above process. The software generates displays and reports which illustrate this and all of the above planning, design, compound data, and graphical representations of compounds.

F. Tools and applications using matrices with memories

1. Tools

The matrix with memory and associated system as described herein is the basic tool that can be used in a multitude of applications, including any reaction that incorporates a functionally specific (i.e. in the reaction) interaction, such as receptor binding. This tool is then combined with existing technologies or can be modified to produce additional tools.

For example, the matrix with memory combination, can be designed as a single analyte test or as a multianalyte test and also as a multiplexed assay that is readily automated. The ability to add one or a mixture of matrices with memories, each with linked or proximate molecule or biological particle to a sample, provides that ability to simultaneously determine multiple analytes and to also avoid multiple pipetting steps. The ability to add a matrix with memory and linked molecules or particles with additional reagents, such as scintillants, provides the ability to multiplex assays.

As discussed herein, in one preferred embodiment the matrices are particulate and include adsorbed, absorbed, or otherwise linked or proximate, molecules, such as peptides or oligonucleotides, or biological particles, such as cells. Assays using such particulate memories with matrices may be conduced "on bead" or "off bead". On bead assays are suitable for multianalyte assays in which mixtures of matrices with linked molecules are used and screened against a labeled known. Off bead assays

may also be performed; in these instances the identity of the linked molecule or biological particle must be known prior to cleavage or the molecule or biological particle must be in some manner associated with the memory.

In other embodiments the matrices with memories use matrices that are continuous, such as microplates, and include a plurality of memories, preferably one memory/well. Of particular interest herein are matrices, such as Flash Plates™ [NEN, Dupont], that are coated or impregnated with scintillant or fluophore or other luminescent moiety or combination thereof, modified by including a memory in each well. The resulting matrix with memory is herein referred to as a luminescing matrix with memory. Other formats of interest that can be modified by including a memory in a matrix include the Multiscreen Assay System [Millipore] and gel permeation technology. Again it is noted that the memories may be replaced with or supplemented with engraved code, preferably at the base of each well [outer surface preferred] that is either precoded or added prior to or during use. The memory, in these instances, is then remote from the matrix.

Preferred plates are those that contain a microplate type frame and removable wells or strips. Each well or strip can contain a memory and/or can be engraved with a code.

2. Scintillation proximity assays (SPAs) and scintillant-containing matrices with memories

Scintillation proximity assays are well known in the art [see, e.g., U.S. Patent No. 4,271,139; U.S. Patent No. 4,382,074; U.S. Patent No. 4,687,636; U.S. Patent No. 4,568,649; U.S. Patent No. 4,388,296; U.S. Patent No. 5,246,869; International PCT Application No. WO 94/26413; International PCT Application No. WO 90/03844; European Patent Application No. 0 556 005 A1; European Patent Application No. 0 301 769 A1; Hart et al. (1979) Molec. Immunol. 16:265-267; Udenfriend et al. (1985) Proc. Natl. Acad. Sci. U.S.A. 82:8672-8676; Nelson et al. (1987) Analyt. Biochem 165:287-293; Heath, et al. (1991) Methodol. Surv. Biochem. Anal. 21:193-194; Mattingly et al. (1995) J. Memb. Sci. 98:275-

280; Pernelle (1993) Biochemistry 32:11682-116878; Bosworth et al. (1989) Nature 341:167-168; and Hart et al. (1989) Nature 341:265]. Beads [particles] and other formats, such as plates and membranes have been developed.

SPA assays refer to homogeneous assays in which quantifiable light energy produced and is related to the amount of radioactively labelled products in the medium. The light is produced by a scintillant that is incorporated or impregnated or otherwise a part of a support matrix. The support matrix is coated with a receptor, ligand or other capture molecule that can specifically bind to a radiolabeled analyte, such as a ligand.

a. Matrices for SPA

Typically, SPA uses fluomicrospheres, such as diphenyloxazole-latex, polyacrylamide-containing a fluophore, and polyvinyltoluene [PVT] plastic scintillator beads, and they are prepared for use by adsorbing compounds into the matrix. Also fluomicrospheres based on organic phosphors have been developed. Microplates made from scintillation plastic, such as PVT, have also been used [see, e.g., International PCT Application No. WO 90/03844]. Numerous other formats are presently available, and any format may be modified for use herein by including one or more recording devices.

Typically the fluomicrospheres or plates are coated with acceptor molecules, such as receptors or antibodies to which ligand binds selectively and reversibly. Initially these assays were performed using glass beads containing fluors and functionalized with recognition groups for binding specific ligands [or receptors], such as organic molecules, proteins, antibodies, and other such molecules. Generally the support bodies used in these assays are prepared by forming a porous amorphous microscopic particle, referred to as a bead [see, e.g., European Patent Application No.0 154,734 and International PCT Application No. WO 91/08489]. The bead is formed from a matrix material such as acrylamide, acrylic acid, polymers of styrene, agar, agarose, polystyrene, and other such materials, such as those

set forth above. Cyanogen bromide has been incorporated into the bead into to provide moieties for linkage of capture molecules or biological particles to the surface. Scintillant material is impregnated or incorporated into the bead by precipitation or other suitable method. Alternatively, the matrices are formed from scintillating material [see, e.g., International PCT Application No. WO 91/08489, which is based on U.S. application Serial No. 07/444,297; see, also U.S. Patent No. 5,198,670], such as yttrium silicates and other glasses, which when activated or doped respond as scintillators. Dopants include Mn, Cu, Pb, Sn, Au, Ag, Sm, and Ce. These materials can be formed into particles or into continuous matrices. For purposes herein, the are used to coat, encase or otherwise be in contact with one or a plurality of recording devices.

Assays are conducted in normal assay buffers and requires the use of a ligand labelled with an isotope, such as ^{3}H and ^{125}I, that emits low-energy radiation that is readily dissipated easily an aqueous medium. Because ^{3}H β particles and ^{125}I Auger electrons have average energies of 6 and 35 keV, respectively, their energies are absorbed by the aqueous solutions within very small distances ($\sim$4 μm for ^{3}H β particles and 35 μm for ^{125}I Auger electrons). Thus, in a typical reaction of 0.1 ml to 0.4 ml the majority of unbound labelled ligands will be too far from the fluomicrosphere to activate the fluor. Bound ligands, however, will be in sufficiently close proximity to the fluomicrospheres to allow the emitted energy to activate the fluor and produce light. As a result bound ligands produce light, but free ligands do not. Thus, assay beads emit light when they are exposed to the radioactive energy from the label bound to the beads through the antigen-antibody linkage, but the unreacted radioactive species in solution is too far from the bead to elicit light. The light from the beads will be measured in a liquid scintillation counter and will be a measure of the bound label.

Matrices with memories for use in scintillation proximity assays [SPA] are prepared by associating a memory [or engraved or iprinted code or symbology] with a matrix that includes a scintillant. In the most simple

embodiment, matrix particles with scintillant [fluomicrospheres] are purchased from Amersham, Packard, NE Technologies [(formerly Nuclear Enterprises, Inc.) San Carlos, CA] or other such source and are associated with a memory, such as by including one or more of such beads in a MICROKAN™ microvessel with a recording device. Typically, such beads as purchased are derivatized and coated with selected moieties, such as streptavidin, protein A, biotin, wheat germ agglutinin [WGA], and polylysine. Also available are inorganic fluomicrospheres based on cerium-doped yttrium silicate or polyvinyltoluene (PVT). These contain scintillant and may be coated and derivatized.

Alternatively, small particles of PVT impregnated with scintillant are used to coat recording devices, such as the IPTT-100 devices [Bio Medic Data Systems, Inc., Maywood, NJ; see, also U.S. Patent Nos. 5,422,636, 5,420,579, 5,262,772, 5,252,962, 5,250,962, 5,074,318, and RE 34,936] that have been coated with a protective material, such as polystyrene, TEFLON, a ceramic or anything that does not interfere with the reading and writing EM frequency(ies). Such PVT particles may be manufactured or purchased from commercial sources such as NE TECHNOLOGY, INC. [e.g., catalog # 191A, 1-10 μm particles]. These particles are mixed with agarose or acrylamide, styrene, vinyl or other suitable monomer that will polymerize or gel to form a layer of this material, which is coated on polystyrene or other protective layer on the recording device. The thickness of the layers may be empirically determined, but they must be sufficiently thin for the scintillant to detect proximate radiolabels. To make the resulting particles resistant to chemical reaction they may be coated with polymers such as polyvinyltoluene or polystyrene, which can then be further derivatized for linkage and/or synthesis of molecules and biological particles. The resulting beads are herein called luminescening matrices with memories, and when used in SPA formats are herein referred to as scintillating matrices with memories.

The scintillating matrices with memories beads can be formed by manufacturing a bead containing a recording device and including scintillant, such as 2,5-diphenyloxazole [PPO] and/or 1,4-bis-[5-phenyl-(oxazolyl)]benzene [POPOP] as a coating. These particles or beads are then coated with derivatized polyvinyl benzene or other suitable matrix on which organic synthesis, protein synthesis or other synthesis can be performed or to which organic molecules, proteins, nucleic acids, biological particles or other such materials can be attached. Attachment may be effected using any of the methods known to those of skill in the art, including methods described herein, and include covalent, non-covalent, direct and indirect linkages.

Alternatively or additionally, each bead may be engraved with a code. Preferably the beads are of such geometry that they can be readily oriented for reading.

Molecules, such as ligands or receptors or biological particles are covalently coupled thereto, and their identity is recorded in the memory. Alternatively, molecules, such as small organics, peptides and oligonucleoties, are synthesized on the beads as described herein so that history of synthesis and/or identity of the linked molecule is recorded in the memory. The resulting matrices with memory particles with linked molecules or biological particles may be used in any application in which SPA is appropriate. Such applications, include, but are not limited to: radioimmunoassays, receptor binding assays, enzyme assays and cell biochemistry assays.

For use herein, the beads, plates and membranes are either combined with a recording device or a plurality of devices, or the materials used in preparing the beads, plates or membranes is used to coat, encase or contact a recording device and/or engraved with a code. Thus, microvessels [MICROKANS™] containing SPA beads coated with a molecule or biological particle of interest; microplates impregnated with or coated with scintillant,

and recording devices otherwise coated with, impregnated with or contacted with scintillant are provided.

To increase photon yield and remove the possibility of loss of fluor, derivatized fluomicrospheres based on yttrium silicate, that is doped selectively with rare earth elements to facilitate production of light with optimum emission characteristics for photomultipliers and electronic circuitry have been developed [see, e.g., European Patent Application No. 0 378 059 B1; U.S. Patent No. 5,246,869]. In practice, solid scintillant fibers, such as cerium-loaded glass or based on rare earths, such as yttrium silicate, are formed into a matrix. The glasses may also include activators, such as terbium, europium or lithium. Alternatively, the fiber matrix may be made from a scintillant loaded polymer, such as polyvinyltoluene. Molecules and biological particles can be adsorbed to the resulting matrix.

For use herein, these fibers may be combined in a microvessel with a recording device [i.e., to form a MICROKAN™]. Alternatively, the fibers are used to coat a recording device or to coat or form a microplate containing recording devices in each well. The resulting combinations are used as supports for synthesis of molecules or for linking biological particles or molecules. The identity and/or location and/or other information about the particles is encoded in the memory and the resulting combinations are used in scintillation proximity assays.

Scintillation plates [e.g., FlashPlates™, NEN Dupont, and other such plates] and membranes have also been developed [see, Mattingly et al. (1995) J. Memb. Sci. 98:275-280] that may be modified by including a memory for use as described herein. The membranes, which can contain polysulfone resin M.W. 752 kD, polyvinylpyrrolidone MW 40 kDA, sulfonated polysulfone, fluor, such as *p*-bis-*o*-methylstyrylbenzene, POP and POPOP, may be prepared as described by Mattingly, but used to coat, encase or contact a recording device. Thus, instead of applying the polymer solution to a glass plate the polymer solution is applied to the recording

device, which, if need is pre-coated with a protective coating, such as a glass, TEFLON or other such coating.

Further, as shown in the Examples, the recording device may be coated with glass, etched and the coated with a layer of scintillant. The scintillant may be formed from a polymer, such as polyacrylamide, gelatin, agarose or other suitable material, containing fluophors, a scintillation cocktail, FlexiScint [Packard Instrument Co., Inc., Downers Grove, IL] NE Technology beads [see, e.g., U.S. Patent No. 4,588,698 for a description of the preparation of such mixtures]. Alternatively, microplates that contain recording devices in one or more wells may be coated with or impregnated with a scintillant or microplates containing scintillant plastic may be manufactured with recording devices in each well. If necessary, the resulting bead, particle or continuous matrix, such as a microplate, may be coated with a thin layer polystyrene, TEFLON or other suitable material. In all embodiments it is critical that the scintillant be in sufficient proximity to the linked molecule or biological particle to detect proximate radioactivity upon interaction of labeled molecules or labeled particles with the linked molecule or biological particle.

The resulting scintillating matrices may be used in any application for which scintillation proximity assays are used. These include, ligand identification, single assays, multianalyte assays, including multi-ligand and multi-receptor assays, radioimmunoassays [RIAs], enzyme assays, and cell biochemistry assays [see, e.g., International PCT Application No. WO 93/19175, U.S. Patent No. 5,430,150, Whitford et al. (1991) Phyto-chemical Analysis 2:134-136; Fenwick et al. (1994) Anal. Proc. Including Anal. Commun. 31:103-106; Skinner et al. (1994) Anal. Biochem. 223:259-265; Matsumura et al. (1992) Life Sciences 51:1603-1611; Cook et al. (1991) Structure and Function of the Aspartic Proteinases, Dunn, ed., Penum Press, NY, pp. 525-528; Bazendale et al. in (1990) Advances in Prostaglandin, Thromboxane and Leukotriene Research, Vol. 21, Samuelsson et al., eds., Raven Press, NY, pp 302-306].

b. Assays

(1) Receptor Binding Assays

Scintillating matrices with memories beads can be used, for example, in assays screening test compounds as agonists or antagonists of receptors or ion channels or other such cell surface protein. Test compounds of interest are synthesized on the beads or linked thereto, the identity of the linked compounds is encoded in the memory either during or following synthesis, linkage or coating. The scintillating matrices with memories are then incubated with radiolabeled [^{125}I, ^{3}H, or other suitable radiolabel] receptor of interest and counted in a liquid scintillation counter. When radiolabeled receptor binds to any of the structure(s) synthesized or linked to the bead, the radioisotope is in sufficient proximity to the bead to stimulate the scintillant to emit light. In contrast By contrast, if a receptor does not bind, less or no radioactivity is associated with the bead, and consequently less light is emitted. Thus, at equilibrium, the presence of molecules that are able to bind the receptor may be detected. When the reading is completed, the memory in each bead that emits light [or more light than a control] queried and the host computer, decoder/encoder, or scanner can interpret the memory in the bead and identify the active ligand.

(a) Multi-ligand assay

Mixtures of scintillating matrices with memories with a variety of linked ligands, which were synthesized on the matrices or linked thereto and their identities encoded in each memory, are incubated with a single receptor. The memory in each light-emitting scintillating matrix with memory is queried and the identity of the binding ligand is determined.

(b) Multi-receptor assays

Similar to conventional indirect or competitive receptor binding assays that are based on the competition between unlabelled ligand and a fixed quantity of radiolabeled ligand for a limited number of binding sites, the scintillating matrices with memories permit the simultaneous screening of a number of ligands for a number of receptor subtypes.

Mixtures of receptor coated beads [one receptor type/per bead; each memory encoded with the identity of the linked receptor] are reacted with labeled ligands specific for each receptor. After the reaction has reached equilibrium, all beads that emit light are reacted with a test compound. Beads that no longer emit light are read.

For example receptor isoforms, such as retinoic acid receptor isoforms, are each linked to a different batch of scintillating matrix with memory beads, and the identity of each isoform is encoded in the memories of linked matrices. After addition of the radiolabeled ligand(s), such as ^{3}H-retinoic acid, a sample of test compounds [natural, synthetic, combinatorial, etc.] is added to the reaction mixture, mixed and incubated for sufficient time to allow the reaction to reach equilibrium. The radiolabeled ligand binds to its receptor, which has been covalently linked to the bead and which the emitted short range electrons will excite the fluophor or scintillant in the beads, producing light. When unlabelled ligand from test mixture is added, if it displaces the labeled ligand it will diminish or stop the fluorescent light signal. At the end of incubation period, the tube can be measured in a liquid scintillation counter to demonstrate if any of the test material reacted with receptor family. Positive samples [reduced or no fluorescence] will be further analyzed for receptor subtyping by querying their memories with the RF detector. In preferred embodiments, each bead will be read and with a fluorescence detector and RF scanner. Those that have a reduced fluorescent signal will be identified and the linked receptor determined by the results from querying the memory.

The same concept can be used to screen for ligands for a number of receptors. In one example, FGF receptor, EGF receptor, and PDGF receptor are each covalently linked to a different batch of scintillating matrix with memory beads. The identity of each receptor is encoded in each memory. After addition of the ^{125}I-ligands [^{125}I-FGF, ^{125}I-EGF, and ^{125}I-PDGF] a sample of test compounds [natural, synthetic, combinatorial, etc.] is added to the tube containing ^{125}I-ligand-receptor-beads, m mixed and incubated for

sufficient time to allow the reaction to reach equilibrium. The radiolabeled ligands bind to their respective receptors receptor that been covalently linked to the bead. By virtue of proximity of the label to the bead, the emitted short range electrons will excite the fluophor in the beads. When unlabelled ligand from test mixture is added, if it displaces the any of the labeled ligand it will diminish or stop the fluorescent signal. At the end of incubation period, the tube can be measured in a liquid scintillation counter to demonstrate if any of the test material reacted with the selected receptor family. Positive samples will be further analyzed for receptor type by passing the resulting complexes measuring the fluorescence of each bead and querying the memories by exposing them to RF or the selected EM radiation. The specificity of test ligand is determined by identifying beads with reduced fluorescence that and determining the identity of the linked receptor by querying the memory.

(c) Other formats

Microspheres, generally polystyrene typically about 0.3 μm - 3.9 μm, are synthesized with scintillant inside can either be purchased or prepared by covalently linking scintillant to the monomer prior to polymerization of the polystyrene or other material. They can then be derivatized [or purchased with with chemical functional groups], such as -COOH, and $-CH_2OH$. Selected compounds or libraries are synthesized on the resulting microspheres linked via the functional groups, as described herein, or receptor, such as radiolabeled receptor, can be coated on the microsphere. The resulting "bead" with linked compounds, can used in a variety of SPA and related assays, including immunoassays, receptor binding assays, protein:protein interaction assays, and other such assays in which the ligands linked to the scintillant-containing microspheres are reacted with memories with matrices that are coated with a selected receptor.

For example, ^{125}I-labeled receptor is passively coated on the memory with matrix and then mixed with ligand that is linked to a the scintillant-containing microspheres. Upon binding the radioisotope into is brought into

close proximity to the scintillant in which effective energy transfer from the β particle will occur, resulting in emission of light.

Alternatively, the memory with matrix [containing scintillant] can also be coated with ^{3}H-containing polyer on which the biological target [i.e., receptor, protein, antibody, antigen] can be linked [via adsorption or via a functional group]. Binding of the ligand brings the scintillant into close proximity to the label, resulting in light emission.

(2) Cell-based Assays

Cell-based assays, which are fundamental for understanding of the biochemical events in cells, have been used with increasing frequency in biology, pharmacology, toxicology, genetics, and oncology [see, e.g., Benjamin et al. (1992) Mol. Cell. Biol. 12:2730-2738] Such cell lines may be constructed or purchased [see, e.g., the Pro-Tox Kit available from Xenometrix, Boulder CO; see, also International PCT Application No. WO 94/7208 cell lines]. Established cell lines, primary cell culture, reporter gene systems in recombinant cells, cells transfected with gene of interest, and recombinant mammalian cell lines have been used to set up cell-based assays. For example Xenometrix, Inc. [Boulder, CO.] provides kits for screening compounds for toxicological endpoints and metabolic profiles using bacteria and human cell lines. Screening is effected by assessing activation of regulatory elements of stress genes fused to reporter genes in bacteria, human liver or colon cell lines and provide information on the cytotoxicity and permeability of test compounds.

In any drug discovery program, cell-based assays offer a broad range of potential targets as well as information on cytotoxicity and permeability. The ability to test large numbers of compounds quickly and efficiently provides a competitive advantage in pharmaceutical lead identification.

High throughput screening with cell-based assays is often limited by the need to use separation, wash, and disruptive processes that compromise the functional integrity of the cells and performance of the assay. Homogeneous or mix-and-measure type assays simplify investigation of

various biochemical events in whole cells and have been developed using scintillation microplates [see, e.g., International PCT Application No. WO 94/26413, which describes scintillant plates that are adapted for attachment and/or growth of cells and proximity assays using such cells]. In certain embodiment herein, cell lines such as those described in International PCT Application No. WO 94/17208 are be plated on scintillant plates, and screened against compounds synthesized on matrices with memories. Matrices with memories encoded with the identity of the linked molecule will be introduced into the plates, the linkages cleaved and the effects of the compounds assessed. Positive compounds will be identified by querying the associated memory.

The scintillant base plate is preferably optically transparent to selected wavelengths that allow cells in culture to be viewed using an inverted phase contrast microscope, and permit the material to transmit light at a given wavelength with maximum efficiency. In addition the base retains its optical properties even after exposure to incident beta radiation from radioisotopes as well as under stringent radiation conditions required for sterilization of the plates. The base plate can be composed of any such optically transparent material containing scintillant, e.g., a scintillant glass based on lanthanide metal compounds. Typically, the base plate is composed of any plastic material, generally formed from monomer units that include phenyl or naphthyl moieties in order to absorb incident radiation energy from radionuclides which are in close proximity with the surface. Preferably the plastic base plate is composed of polystyrene or polyvinyltoluene, into which the scintillant is incorporated. The scintillant includes, but is not limited to: aromatic hydrocarbons such as p-terphenyl, p-quaterphenyl and their derivatives, as well as derivatives of the oxazoles and 1,3,4-oxadiazoles, such as 2-(4-t-butylphenyl)-5-(4-biphenyl)-1,3,4-oxadiazole and 2,5-diphenyloxazole. Also included in the polymeric composition may be a wavelength shifter such as 1,4-bis(5-phenyl-2-oxazolyl)benzene, 9,10-diphenylanthracene, 1,4-bis(2-methylstyryl)-benzene,

and other such compounds. The function of the wavelength shifter is to absorb the light emitted by the scintillant substance and re-emit longer wavelength light which is a better match to the photo-sensitive detectors used in scintillation counters. Other scintillant substances and polymer bodies containing them are known to those of skill in this art [see, e.g., European Patent Application No. 0 556 005 A1].

The scintillant substances can be incorporated into the plastic material of the base by a variety of methods. For example, the scintillators may be dissolved into the monomer mix prior to polymerization, so that they are distributed evenly throughout the resultant polymer. Alternatively the scintillant substances may be dissolved in a solution of the polymer and the solvent removed to leave a homogeneous mixture. The base plate of disc may be bonded to the main body of the well or array of wells, which itself may be composed of a plastic material including polystyrene, polyvinyltoluene, or other such polymers. In the case of the multi-well array, the body of the plate may be made opaque, i.e., non-transparent and internally reflective, in order to completely exclude transmission of light and hence minimize "cross-talk." This is accomplished by incorporating into the plastic at the polymerization stage a white dye or pigment, for example, titanium dioxide. Bonding of the base plate to the main body of the device can be accomplished by any suitable bonding technique, for example, heat welding, injection molding or ultrasonic welding.

For example, a 96-well plate is constructed to the standard dimensions of 96-well microtiter plates 12.8 cm x 8.6 cm x 1.45 cm with wells in an array of 8 rows of 12 wells each. The main body of the plate is constructed by injection molding of polystyrene containing a loading of white titanium oxide pigment at 12%. At this stage, the wells of the microtiter plate are cylindrical tubes with no closed end. A base plate is formed by injection molding of polystyrene containing 2-(4-t-butylphenyl)-5-(4-biphenyl)-1,3,4-oxadiazole (2%) and 9,10-diphenylanthracene (0.5%). The base plate has been silk screen printed with a grid array to further

reduce crosstalk. The base plate is then fused in a separate operation to the body by ultrasonic welding, such that the grid array overlies the portions of the microtiter plate between the wells.

A 24-well device is constructed to the dimensions 12.8 x 8.6 x 1.4 cm with 24 wells in an array of 4 rows of 6 wells. The main body of the plate [not including the base of each well] is constructed by injection molding of polystyrene containing 12% white titanium oxide pigment. The base 24 of each well is injection molded with polystyrene containing 2-(4-t-butylphenyl)-5-(4-biphenylyl)-1,3,4-oxadizaole [2%] and 9,10-diphenylanthracene [0.5%]. The heat from the injected base plastic results in fusion to the main body giving an optically transparent base to the well.

The plates may contain multiple wells that are continuous or that are each discontinuous from the other wells in the array, or they may be single vessels that have, for example, an open top, side walls and an optically transparent scintillant plastic base sealed around the lower edge of the side walls.

In another format the plate, is a single well or tube. The tube may be constructed from a hollow cylinder made from optically transparent plastic material and a circular, scintillant containing, plastic disc. The two components are welded together so as to form a single well or tube suitable for growing cells in culture. As in the plate format, bonding of the circular base plate to the cylindrical portion is achieved by any conventional bonding technique, such as ultrasonic welding. The single well or tube may be any convenient size, suitable for scintillation counting. In use, the single well may either be counted as an insert in a scintillation vial, or alternatively as an insert in a scintillation vial, or alternatively as an insert in a multi-well plate of a flat bed scintillation counter. In this latter case, the main body of the multi-well plate would need to be opaque for reasons given earlier.

The various formats are selected according to use. They may be used for growing cells and studying cellular biochemical processes in living cells or cell fragments. The 96-well plate is a standard format used in experimental cell biology and one that is suitable for use in a flat bed scintillation counter [e.g., Wallac Microbeta or Packard Top Count]. In the multi-well format, it is an advantage to be able to prevent "cross talk" between different wells of the plate that may be used for monitoring different biological processes using different amounts or types of radioisotope. Therefore the main body of the plate can be made from opaque plastic material. The 24-well plate format is commonly used for cell culture. This type of plate is also suitable for counting in a flat bed scintillation counter. The dimensions of the wells will be larger.

As an alternative format, the transparent, scintillant containing plastic disc is made to be of suitable dimensions so as to fit into the bottom of a counting vessel. The counting vessel is made from non-scintillant containing material such as glass or plastic and should be sterile in order to allow cells to grow and the corresponding cellular metabolic processes to continue. Cells are first cultured on the disc, which is then transferred to the counting vessel for the purposes of monitoring cellular biochemical processes.

The culture of cells on the scintillation plastic base plate of the wells (or the disc) involves the use of standard cell culture procedures, e.g., cells are cultured in a sterile environment at 37° C in an incubator containing a humidified 95% air/5% CO_2 atmosphere. Various cell culture media may be used including media containing undefined biological fluids such as fetal calf serum, or media which is fully defined and serum-free. For example, MCDB 153 is a selective medium for the culture of human keratinocytes [Tsao et al. (1982) J. Cell. Physiol. 110:219-229].

These plates are suitable for use with any adherent cell type that can be cultured on standard tissue culture plasticware, including culture of primary cells, normal and transformed cells derived from recognized sources species and tissue sources. In addition, cells that have been transfected

with the recombinant genes may also be cultured using the invention. There are established protocols available for the culture of many of these diverse cell types [see, e.g., Freshney et al. (1987) Culture of Animal Cells: A Manual of Basic Technique, 2nd Edition, Alan R. Liss Inc.]. These protocols may require the use of specialized coatings and selective media to enable cell growth and the expression of specialized cellular functions.

The scintillating base plate or disc, like all plastic tissue culture ware, requires surface modification in order to be adapted for the attachment and/or growth of cells. Treatment can involves the use of high voltage plasma discharge, a well established method for creating a negatively charged plastic surface [see, e.g., Amstein et al. (1975) J. Clinical Microbiol. 2:46-54]. Cell attachment, growth and the expression of specialized functions can be further improved by applying a range of additional coatings to the culture surface of the device. These can include: (i) positively or negatively charged chemical coatings such as poly-lysine or other biopolymers [McKeehan et al. (1976) J. Cell Biol. 71:727-734 (1976)]; (ii) components of the extracellular matrix including collagen, laminin, fibronectin [see, e.g., Kleinman et al. (1987) Anal. Biochem. 166:1-13]; and (iii) naturally secreted extracellular matrix laid down by cells cultured on the plastic surface [Freshney et al. et al. (1987) Culture of Animal Cells: A Manual of Basic Technique, 2nd Edition, Alan R. Liss Inc.]. Furthermore, the scintillating base plate may be coated with agents, such as lectins, or adhesion molecules for attachment of cell membranes or cell types that normally grow in suspension. Methods for the coating of plasticware with such agents are known [see, e.g., Boldt et al. (1979) J. Immunol. 123:808].

In addition, the surface of the scintillating layer may be coated with living or dead cells, cellular material, or other coatings of biological relevance. The interaction of radiolabeled living cells, or other structures with this layer can be monitored with time allowing processes such as binding, movement to or from or through the layer to be measured.

Virtually all types of biological molecules can be studied. A any molecule or complex of molecules that interact with the cell surface or that can be taken up, transported and metabolized by the cells, can be examined using real time analysis. Examples of biomolecules will include receptor ligands, protein and lipid metabolite precursors (e.g., amino acids, fatty acids), nucleosides and any molecule that can be radiolabeled. This would also include ions such as calcium, potassium, sodium and chloride, that are functionally important in cellular homeostasis, and which exist as radioactive isotopes. Furthermore, viruses and bacteria and other cell types, which can be radiolabeled as intact moieties, can be examined for their interaction with monolayer adherent cells grown in the scintillant well format.

The type of radioactive isotope that can be used with this system will typically include any of the group of isotopes that emit electrons having a mean range up to 2000 μm in aqueous medium. These will include isotopes commonly used in biochemistry such as [^{3}H], [^{125}I], [^{14}C], [^{35}S], [^{45}Ca], [^{33}p], and [^{32}p], but does not preclude the use of other isotopes, such as [^{55}Fe], [^{109}Cd] and [^{51}Cr] that also emit electrons within this range. The wide utility of the invention for isotopes of different emission energy is due to the fact that the current formats envisaged would allow changes to the thickness of the layer containing a scintillant substance, thereby ensuring that all the electron energy is absorbed by the scintillant substance. Furthermore, cross-talk correction software is available which can be utilized with all high energy emitters. Applications using these plates include protein synthesis, Ca^{2+} transport, receptor-ligand binding, cell adhesion, sugar transport and metabolism, hormonal stimulation, growth factor regulation and stimulation of motility, thymidine transport, and protein synthesis.

For use in accord with the methods herein, the scintillant plates can include a memory in each well, or alternatively, memory with matrix-linked compounds will be added to each well. The recording device with memory may be impregnated or encased or placed in wells of the plate, typically

during manufacture. In preferred embodiments, however, the memories are added to the wells with adsorbed or linked molecules.

In one embodiment, matrices with memories with linked molecules are introduced into scintillant plates in which cells have been cultured [see, e.g., International PCT Application No. WO 94/26413]. For example, cells will be plated on the transparent scintillant base 96-well microplate that permits examination of cells in culture by inverted phase contrast microscope and permits the material to transmit light at a given wavelength with maximum efficiency. Matrices with memories to which test compounds linked by preferably a photocleaveable linker are added to the wells. The identity of each test compound is encoded in the memory of the matrix during synthesis if the compound is synthesized on the matrix with memory or when the compound is linked to the matrix.

Following addition of matrix with memory to the well and release of chemical entities synthesized on the beads by exposure to light or other procedures, the effects of the chemical released from the beads on the selected biochemical events, such as signal transduction, cell proliferation, protein or DNA synthesis, in the cells can be assessed. In this format receptor binding Such events include, but are not limited to: whole cell receptor-ligand binding [agonist or antagonist], thymidine or uridine transport, protein synthesis (using, for example, labeled cysteine, methionine, leucine or proline], hormone and growth factor induced stimulation and motility, and calcium uptake.

In another embodiment, the memories are included in the plates either placed in the plates or manufactured in the wells of the plates. In these formats, the identities of the contents of the well is encoded into the memory. Of course it is understood, that the information encoded and selection of encased or added memories depends upon the selected protocol.

In another format, cells will be plated on the tissue culture plate, after transferring the matrices with memories and release of compounds

synthesized on the beads in the well. Cytostatic, cytotoxic and proliferative effects of the compounds will be measured using colorimetric [MTT, XTT, MTS, Alamar blue, and Sulforhodamine B], fluorimetric [carboxyfluorescein diacetate] , or chemiluminescent reagents [i.e., CytoLiteTM, Packard Instruments, which is used in a homogeneous luminescent assay for cell proliferation, cell toxicity and multi-drug resistance].

For example, cells that have been stably or transiently transfected with a specific gene reporter construct containing an inducible promoter operatively linked to a reporter gene that encodes an indicator protein can be colorimetrically monitored for promoter induction. Cells will be plated on the tissue culture 96-well microtiter plate and after addition of memories with matrices in the wells and release of chemical entities synthesized on the matrices, the effect of the compound released from the beads on the gene expression will be assessed. The Cytosensor Microphysiometer [Molecular Devices] evaluates cellular responses that are mediated by G protein-linked receptors, tyrosine kinase-linked receptors, and ligand-gated ion channels. It measures extracellular pH to assess profiles of compounds assessed for the ability to modulate activities of any of the these cell surface proteins by detecting secretion of acid metabolites as a result of altered metabolic states, particularly changes in metabolic rate. Receptor activation requires use of ATP and other energy resources of the cell thereby leading to increased in cellular metabolic rate. For embodiments herein, the memories with matrices, particularly those modified for measuring pH, and including linked test compounds, can be used to track and identify the added test compound added and also to detect changes in pH, thereby identifying linked molecules that modulate receptor activities.

3. Immunoassays and immunodiagnostics

The combinations and methods provided herein represent major advances in immunodiagnotics. Immunoassays [such as ELISAs, RIAs and EIAs (enzyme immunoassays)] are used to detect and quantify antigens or antibodies or to screen compounds.

a. Immunoassays

Immunoassays detect or quantify very small concentrations of analytes in biological samples. Many immunoassays use solid supports in which antigen or antibody is covalently, non-covalently, or otherwise, such as via a linker, attached to a solid support matrix. The support-bound antigen or antibody is then used as an analyte in the assay. As with nucleic acid analysis, the resulting antibody-antigen complexes or other complexes, depending upon the format used, rely on radiolabels or enzyme labels to detect such complexes.

The use of antibodies to detect and/or quantitate reagents ["antigens"] in blood or other body fluids has been widely practiced for many years. Two methods have been most broadly adopted. The first such procedure is the competitive binding assay, in which conditions of limiting antibody are established such that only a fraction [usually 30-50%] of a labeled [e.g., radioisotope, fluophore or enzyme] antigen can bind to the amount of antibody in the assay medium. Under those conditions, the addition of unlabeled antigen [e.g., in a serum sample to be tested] then competes with the labeled antigen for the limiting antibody binding sites and reduces the amount of labeled antigen that can bind. The degree to which the labeled antigen is able to bind is inversely proportional to the amount of unlabeled antigen present. By separating the antibody-bound from the unbound labeled antigen and then determining the amount of labeled reagent present, the amount of unlabeled antigen in the sample [e.g., serum] can be determined.

As an alternative to the competitive binding assay, in the labeled antibody, or "immunometric" assay [also known as "sandwich" assay], an antigen present in the assay fluid is specifically bound to a solid substrate and the amount of antigen bound is then detected by a labeled antibody [see, e.g., Miles et al. (1968) Nature 29:186-189; U.S. Patent No. 3,867,517; U.S. Patent No. 4,376,110]. Using monoclonal antibodies two-site immunometric assays are available [see, e.g.,U.S. Patent No.

4,376,110]. The "sandwich" assay has been broadly adopted in clinical medicine. With increasing interest in "panels" of diagnostic tests, in which a number of different antigens in a fluid are measured, the need to carry out each immunoassay separately becomes a serious limitation of current quantitative assay technology.

Some semi-quantitative detection systems have been developed [see, e.g., Buechler et al. (1992) Clin. Chem. 38:1678-1684; and U.S. Patent No. 5,089,391] for use with immunoassays, but no good technologies yet exist to carefully quantitate a large number of analytes simultaneously [see, e.g., Ekins et al. (1990) J. Clin. Immunoassay 13:169-181] or to rapidly and conveniently track, identify and quantitate detected analytes.

The methods and memories with matrices provided herein provide a means to quantitate a large number of analytes simultaneously and to rapidly and conveniently track, identify and quantitate detected analytes.

b. **Multianalyte immunoassays**

The combinations of matrix with memories provided herein permits the simultaneous assay of large numbers of analytes in any format. In general, the sample that contains an analyte, such as a ligand or any substance of interest, to be detected or quantitated, is incubated with and bound to a protein, such as receptor or antibody, or nucleic acid or other molecule to which the analyte of interest binds. In one embodiment, the protein or nucleic acid or other molecule to which the analyte of interest binds has been linked to a matrix with memory prior to incubation; in another embodiment, complex of analyte or ligand and protein, nucleic acid or other molecule to which the analyte of interest binds is linked to the matrix with memory after the incubation; and in a third embodiment, incubation to form complexes and attachment of the complexes to the matrix with memory are simultaneous. In any embodiment, attachment is effected, for example, by direct covalent attachment, by kinetically inert attachment, by noncovalent linkage, or by indirect linkage, such as through a second binding reaction [i.e., biotin-avidin, Protein A-antibody, antibody-

hapten, hybridization to form nucleic acid duplexes of oligonucleotides, and other such reactions and interactions]. The complexes are detected and quantitated on the solid phase by virtue of a label, such as radiolabel, fluorescent label, luminophore label, enzyme label or any other such label. The information that is encoded in the matrix with memory depends upon the selected embodiment. If, for example, the target molecule, such as the protein or receptor is bound to the solid phase, prior to complexation, the identity of the receptor and/or source of the receptor may be encoded in the memory in the matrix.

For example, the combinations provided herein are particularly suitable for analyses of multianalytes in a fluid, and particularly for multianalyte immunoassays. In one example, monoclonal antibodies very specific for carcinoembryonic antigen [CEA], prostate specific antigen [PSA], CA-125, alphafetoprotein [AFP], TGF-β, IL-2, IL-8 and IL-10 are each covalently attached to a different batch of matrices with memories using well-established procedures and matrices for solid phase antibody assays. Each antibody-matrix with memory complex is given a specific identification tag, as described herein.

A sample of serum from a patient to be screened for the presence or concentration of these antigens is added to a tube containing two of each antibody-matrix with memory complex [a total of 16 beads, or duplicates of each kind of bead]. A mixture of monoclonal antibodies, previously conjugated to fluorescent dyes, such as fluorescein or phenyl-EDTA-Eu chelate, reactive with different epitopes on each of the antigens is then added. The tubes are then sealed and the contents are mixed for sufficient time [typically one hour] to allow any antigens present to bind to their specific antibody-matrix with memory-antigen complex to produce antibody-matrix with memory-antigen-labeled antibody complexes. At the end of the time period, these resulting complexes are briefly rinsed and passed through an apparatus, such as that set forth in FIGURE 7, but with an additional light source. As each complex passes through a light source, such as a laser

emitting at the excitation wavelength of fluorescein, about 494 nm, or 340 nm for the Eu chelate complex, its fluorescence is measured and quantitated by reading the emitted photons at about 518 nm for fluorescein or 613 nm for phenyl-EDTA-Eu, and as its identity is determined by the specific signal received by the RF detector. In this manner, eight different antigens are simultaneously detected and quantitated in duplicate.

In another embodiment, the electromagnetically tagged matrices with recorded information regarding linked antibodies can be used with other multianalyte assays, such as those described by Ekins et al. [(1990) J. Clin. Immunoassay 13:169-181; see, also International PCT Applications Nos. 89/01157 and 93/08472, and U.S. Patent Nos. 4,745,072, 5,171,695 and 5,304,498]. These methods rely on the use of small concentrations of sensor-antibodies within a few μm^2 area. Individual memories with matrices, or an array of memories embedded in a matrix are used. Different antibodies are linked to each memory, which is programmed to record the identity of the linked antibody. Alternatively, the antibody can be linked, and its identity or binding sites identified, and the information recorded in the memory. Linkage of the antibodies can be effected by any method known to those of skill in this art, but is preferably effected using cobalt-iminodiacetate coated memories [see, Hale (1995) Analytical Biochem. 231:46-49, which describes means for immobilization of antibodies to cobalt-iminodiacetate resin] mediated linkage particularly advantageous. Antibodies that are revesibly bound to a cobalt-iminodiacetate resin are attached in exchange insert manner when the cobalt is oxidized from the +2 to +3 state. In this state the antibodies are not removed by metal chelating regents, high salt, detergents or chaotropic agents. They are only removed by reducing agents. In addition, since the metal binding site in antibodies is in the C-terminus heavy chain, antibodies so-bound are oriented with the combining site directed away from the resin.

In particular antibodies are linked to the matrices with memories. The matrices are either in particular form or in the form of a slab with an array of

recording devices linked to the matrices or microtiter dish or the like with a recording device in each well. Antibodies are then linked either to each matrix particle or to discrete "microspots" on the slab or in the microtiter wells. In one application, prior to use of these matrices with memories, they are bound to a relatively low affinity anti-idiotype antibody [or other species that specifically recognizes the antibody binding site, such as a single chain antibody or peptidomimetic] labeled with a fluophore [e.g., Texas Red, see, Ekins et al. (1990) J. Clin. Immunoassay 13:169-181] to measure the concentration of and number of available binding sites present on each matrix with memory particle or each microspot, which information is then encoded into each memory for each microspot or each particle. These low affinity antibodies are then eluted, and the matrices can be dried and stored until used.

Alternatively or additionally, the memories in the particles or at each microspot could be programmed with the identity or specificity of the linked antibody, so that after reaction with the test sample and identification of complexed antibodies, the presence and concentration of particular analytes in the sample can be determined. They can be used for multianalyte analyses as described above.

After reaction with the test sample, the matrices with memories are reacted with a second antibody, preferably, although not necessarily, labeled with a different label, such as a different fluophore, such as fluorescein. After this incubation, the microspots or each matrix particle is read by passing the particle through a laser scanner [such as a confocal microscope, see, e.g., Ekins et al. (1990) J. Clin. Immunoassay 13:169-181; see also U.S. Patent No. 5,342,633] to determine the fluorescence intensity. The memories at each spot or linked to each particle are queried to determine the total number of available binding sites, thereby permitting calculation of the ratio of occupied to unoccupied binding sites.

Equilibrium dialysis and modifications thereof has been used to study the interaction of antibody or receptor or other protein or nucleic acid with low molecular weight dialyzable molecules that bind to the antibody or receptor or other protein or nucleic acid. For applications herein, the antibody, receptor, protein or nucleic acid is linked to solid support (matrix with memory) and is incubated with the ligand.

In particular, this method may be used for analysis of multiple binding agents [receptors], linked to matrices with memories, that compete for available ligand, which is present in limiting concentration. After reaction, the matrices with memories linked to the binding agents [receptors] with the greatest amount of bound ligand, are the binding agents [receptors] that have the greatest affinity for the ligand.

The use of matrices with memories also permits simultaneous determination of K_a values of multiple binding agents [receptors] or have multiple ligands. For example, a low concentration of labeled ligand is mixed with a batch of different antibodies bound to matrices with memories. The mixture is flowed through a reader [i.e., a Coulter counter or other such instrument that reads RF and the label] could simultaneously measure the ligand [by virtue of the label] and identity of each linked binding agent [or linked ligand] as the chip is read. After the reaction equilibrium [determined by monitoring progress of the reaction] labeled ligand is added and the process of reading label and the chips repeated. This process is repeated until all binding sites on the binding agent [or ligand] approach saturation, thereby permitting calculation of K_a values and binding sites that were available.

4. Memories with matrices for non-radioactive energy transfer proximity assays

Non-radioactive energy transfer reactions, such as FET or FRET, FP and HTRF assays, are homogeneous luminescence assays based on energy transfer are carried out between a donor luminescent label and an acceptor label [see, e.g., Cardullo et al. (1988) Proc. Natl. Acad. Sci. U.S.A.

85:8790-8794; Peerce et al. (1986) Proc. Natl. Acad. Sci. U.S.A. 83:8092-8096; U.S. Patent No. 4,777,128; U.S. Patent No. 5,162,508; U.S. Patent No. 4,927,923; U.S. Patent No. 5,279,943; and International PCT Application No. WO 92/01225]. The donor label is usually a rare earth metal cryptate, particularly europium trisbipyridine diamine [EuTBP] or terbium trisbipyridine diamine [TbTBP] and an acceptor luminescent, presently fluorescent, label. When the donor is EuTBP, the acceptor is preferably allopycocyanin [APC], allophycocyanin B, phycocyanin C or phycocyanin R, and when the donor is TbTBP, the acceptor is a rhodamine, thiomine, phycocyanin R, phycoerythrocyanin, phycoerythrin C, phycoerythrin B or phycoerythrin R.

Energy transfer between such donors and acceptors is highly efficient, giving an amplified signal and thereby improving the precision and sensitivity of the assay. Within distances characteristic of interactions between biological molecules, the excitation of a fluorescent label (donor) is transferred non radiatively to a second fluorescent label (acceptor). When using europium cryptate as the donor, APC, a phycobiliprotein of 5 kDa, is presently the preferred acceptor because it has high molar absorptivity at the cryptate emission wavelength providing a high transfer efficiency, emission in a spectral range in which the cryptate signal is insignificant, emission that is not quenched by presence of sera, and a high quantum yield. When using Eu^{3+} cryptate as donor, an amplification of emitted fluorescence is obtained by measuring APC emission.

The rare earth cryptates are formed by the inclusion of a luminescence lanthanide ion in the cavity of a macropolycyclic ligand containing 2,2'-biphyridine groups as light absorbers [see, e.g., U.S. Patent No. 5,162,508; U.S. Patent No. 4,927,923; U.S. Patent No. 5,279,943; and International PCT Application No. WO 92/01225]. Preferably the $Eu3^{+}$ trisbypryidine diamine derivative, although the acceptor may be used as the label, is cross-linked to antigens, antibodies, proteins, peptides, and oligonucleotides and other molecules of interest.

For use herein, matrices with memories are prepared that incorporate either the donor or, preferably the acceptor, into or on the matrix. In practice, as with the scintillating matrices with memories, the matrices may be of any format, i.e. particulate, or continuous, and used in any assay described above for the scintillating matrices. For example, the recording device is coated with a protective coating, such as glass or polystyrene. If glass it can be etched. As with preparation of the scintillating matrices with memories, compositions containing the donor or preferably acceptor, such as APC, and typically a polymer or gel, are coated on the recording device or the device is mixed with the composition to produce a fluorescing matrix with memory. To make these matrices resistant to chemical reaction, if needed, they may be coated with polymers such as polyvinylbenzene or polystyrene. Molecules, such as the constituents of combinatorial libraries, are synthesized on the fluorescing matrices with memories, or molecules or biological particles are linked thereto, the identity of the synthesized molecules or linked molecules or biological particles is encoded in memory, and the resulting matrices with memories employed in any suitable assay, including any of those described for the scintillating memories with matrices. In particular, these homogeneous assays using long-lived fluorescence rare earth cryptates and amplification by non radiative energy transfer have been adapted to use in numerous assays including assays employing ligand receptor interaction, signal transduction, transcription factors (protein-protein interaction), enzyme substrate assays and DNA hybridization and analysis [see, Nowak (1993) Science 270:368; see, also, Velculescu et al. (1995) Science 270:484-487, and Schena et al. (1995) Science 270:467-470, which describe methods quantitative and simultaneous analysis of a large number of transcripts that are particularly suited for modification using matrices with memories]. Each of these assays may be modified using the fluorescing matrices with memories provided herein.

For example, a receptor will be labeled with a europium cryptate [where the matrices with memories incorporate, for example

allophycocyanin (APC)] or will be labeled with APC, where the matrices incorporate a europium cryptate. After mixing receptor and mixtures of matrices with different ligands, the mixture is exposed to laser excitation at 337 nm, and, if reaction has occurred, typical signals of europium cryptate and APC over background are emitted. Measurement with an interference filter centered at 665 nm selects the signal of the APC labeled receptor from that of europium cryptate labeled ligand on the beads. If particulate, the memories of matrices that emit at 665, can be queried to identify linked ligands.

5. Other applications using memories with matrices and luminescing memories with matrices

a. Combinatorial libraries and other libraries and screening methododologies

The combinations of matrices with memories are applicable to virtually any synthetic scheme and library preparation and screening protocol. These include, those discussed herein, and also methodologies and devices, such as the Chiron "pin" technology [see, e.g., International PCT application No.WO 94/11388; Geysen et al. (1985) Proc. Natl. Acad. Sci. U.S.A. 82:178; and Geysen et al. (1987) J. Immunol. Meth. 102:259-274] which relies on a support composed of annular synthesis components that have an active surface for synthesis of a modular polymer and an inert support rod that is positioned axially to the annular synthesis components. This pin technology was developed fo rthe simultaneous synthesis of multiple peptides. In particular the peptides are synthesized on polyacrylic acid grafted on the tip of polyethylene pins, typically arranged in a microtiter format. Amino acid coupling is effected by immersing the pins in a microtiter plate. The resulting peptides remain bound to the pins and can be resused.

As provided herein, "pins" may be linked to a memory or recording device, preferably encasing the device, or each pin may be coded and the code and the identity of the associated linked molecule(s) stored in a remote

memory. As a result it will not be necessary to physically array the pins, rather the pins can be removed and mixed or sorted.

Also of interest herein, are DIVERSOMER™ technology libraries produced by simultaneous parallel sythesis schemes for production of nonoligomeric chemical diversity [see, e.g., U.S. Patent No. 5,424,483; Hobbs DeWitt et al. (1994) Drug Devel. Res. 33:116-124; Czarnik et al. (1994) Polym. Prepr. 35:985; Stankovic et al. (1994) in Innovation Perspect. Solid Phase Synth. Collect. Pap., Int. Symp., 3rd Epton, R. (Ed), pp. 391-6; DeWitt et al. (1994) Drug Dev. Res. 33:116-124; Hobbs DeWitt et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:6909-6913]. In this technology a startingmaterial is bonded to a solid phase, such as a matrix metraial, and is sbusequently treated with reagents in a stepwise fashion. Because the products are linked to the solid suport, multistep syntheses can be automated and multiple reactions can be performed simultaneously to produce libraries of small molecules. This technology can be readily improved by combining the matrices with memories or encoding the matrix supports in accord with the methods herein.

The matrices with memories, either those with memories in proximity or those in which the matrix includes a code stored in a remote memory, can be used in virtually any combinatorial library protocol. These protocols or methodologies and libraries, include but are not limited to those described in any of following references: Zuckermann et al. (1994) J. Med. Chem. 37:2678; Martin et al. (1995) J. Med. Chem. 38:1431; Campbell et al. (1995) J. Am. Chem. Soc. 117:5381; Salmon et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:11708; Patek et al. (1994) Tetrahedron Lett. 35:9169; Patek et al. (1995) Tetrahedron Lett. 36:2227; Hobbs DeWitt et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:6906; Baldwin et al. (1995) J. Am. Chem. Soc. 117:5588; and any others.

b. **Multiplexed or coupled protocols in which the synthesis steps [the chemistry] is coupled to subsequent uses of the synthesized molecules**

Multiplexed or multiple step processes in which compounds are synthesized and then assayed without any intermediate identification steps are provided herein. Since the memories with matrices permit identification of linked or proximate or associated molecules or biological particles, there is no need to identify such molecules or biological particles during any preparative and subsequent assaying steps or processing steps. Thus, the chemistry [synthesis] can be directly coupled to the biology [assaying, screening or any other application disclosed herein]. For purposes herein this coupling is referred to as multiplexing.

Thus, high speed synthesis can be coupled to high throughput screening protocols.

G. **Applications of the memories with matrices and luminescing matrices with memories in combinatorial syntheses and preparation of libraries**

Libraries of diverse molecules are critical for identification of new pharmaceuticals. A diversity library has three components: solid support matrix, linker and synthetic target. The support is a matrix material as described herein that is stable to a wide range of reaction conditoins and solvents; the linker is selectively cleavable and does not leave a functionalized appendage on the synthetic target; and the target is synthesized in high yield and purity. For use herein, the diversity library further includes a memory or recording device in combination with the support matrix. The memory is linked, encased, in proximity with or otherwise associate with each matrix particle, whereby the identify of synthesized targets is written into the memory.

The matrices with memories are linked to molecules and particles that are components of libraries to electronically tagged combinatorial libraries. Particularly preferred libraries are the combinatorial libraries that containing

matrices with memories that employ radio frequencies for reading and writing.

1. Oligomer and polypeptide libraries

a. Bio-oligomer libraries

One exemplary method for generating a library [see, U.S. Patent No. 5,382,513] involves repeating the steps of (1) providing at least two aliquots of a solid phase support; separately introducing a set of subunits to the aliquots of the solid phase support; completely coupling the subunit to substantially all sites of the solid phase support to form a solid phase support/new subunit combination, assessing the completeness of coupling and if necessary, forcing the reaction to completeness; thoroughly mixing the aliquots of solid phase support/new subunit combination; and, after repeating the foregoing steps the desired number of times, removing protecting groups such that the bio-oligomer remains linked to the solid phase support. In one embodiment, the subunit may be an amino acid, and the bio-oligomer may be a peptide. In another embodiment, the subunit may be a nucleoside and the bio-oligomer may be an oligonucleotide. In a further embodiment, the nucleoside is deoxyribonucleic acid; in yet another embodiment, the nucleoside is ribonucleic acid. In a further embodiment, the subunit may be an amino acid, oligosaccharide, oligoglycosides or a nucleoside, and the bio-oligomer may be a peptide-oligonucleotide chimera or other chimera. Each solid phase support is attached to a single bio-oligomer species and all possible combinations of monomer [or multimers in certain embodiments] subunits of which the bio-oligomers are composed are included in the collection.

In practicing this method herein, the support matrix has a recording device with programmable memory, encased, linked or otherwise attached to the matrix material, and at each step in the synthesis the support matrix to which the nascent polymer is attached is programmed to record the identity of the subunit that is added. At the completion of synthesis of each biopolymer, the resulting biopolymers linked to the supports are mixed.

After mixing an acceptor molecule or substrate molecule of interest is added. The acceptor molecule is one that recognizes and binds to one or more solid phase matrices with memory/bio-oligomer species within the mixture or the substrate molecule will undergo a chemical reaction catalyzed by one or more solid phase matrix with memory/bio-oligomer species within the library. The resulting combinations that bind to the acceptor molecule or catalyze reaction are selected. The memory in the matrix-memory combination is read and the identity of the active bio-oligomer species is determined.

b. Split Bead Sequential Syntheses

Various schemes for split bead syntheses of polymers [FIGURE 1], peptides [FIGURE 2], nucleic acids [FIGURE 3] and organic molecules based on a pharmacophore monomer [FIGURE 4] are provided. Selected matrices with memory particles are placed in a suitable separation system, such as a funnel [see, FIGURE 5]. After each synthetic step, each particle is scanned [i.e., read] as it passes the RF transmitter, and information identifying the added component or class of components is stored in memory. For each type of synthesis a code can be programmed [i.e., a 1 at position 1,1 in the memory could, for example, represent alanine at the first position in the peptide]. A host computer or decoder/encoder is programmed to send the appropriate signal to a transmitter that results in the appropriate information stored in the memory [i.e. for alanine as amino acid 1, a 1 stored at position 1,1]. When read, the host computer or decoder/encoder can interpret the signal read from and transmitted from the memory.

In an exemplary embodiment, a selected number of beads [i.e., particulate matrices with memories [matrix particles linked to recording devices], typically at least 10^3, more often 10^4, and desirably at least 10^5 or more up to and perhaps exceeding 10^{15}, are selected or prepared. The beads are then divided into groups, depending upon the number of choices for the first component of the molecule. They are divided into a number of containers equal to or less than [for pooled screening, nested libraries or the other such

methods] the number of choices. The containers can be microtiter wells, Merrifield synthesis vessels, columns, test tubes, gels, etc. The appropriate reagents and monomer are added to each container and the beads in the first container are scanned with electromagnetic with radiation, preferably high frequency radio waves, to transmit information and encode the memory to identify the first monomer. The beads in the second container are so treated. The beads are then combined and separated according to the combinatorial protocol, and at each stage of added monomer each separate group is labeled by inputting data specific to the monomer. At the end of the synthesis protocol each bead has an oligomer attached and information identifying the oligomer stored in memory in a form that can be retrieved and decoded to reveal the identity of each oligomer.

An 8-member decapeptide library was designed, synthesized, and screened against an antibody specifically generated against one of the library members using the matrices with memories. Rapid and clean encoding and decoding of structural information using radio frequency signals, coupling of combinatorial chemical synthesis to biological assay protocols, and potential to sense and measure biodata using suitable biosensors, such as a temperature thermistor or pH electrode, embedded within the devices have been demonstrated. The "split and pool" method [see, e.g., Furka et al. (19910 Int. J. Pept. Protein Res. 37:487-493; Lam et al. (1991) Nature 354:82-84; and Sebestyén et al. (1993) Bioorg. Med. Chem. Lett. 3:413-418] was used to generate the library. An ELISA [see e.g., Harlow et al. (1988) Antibodies, a laboratory manual, Cold Spring Harbor, NY] was used to screen the library for the peptide specific for the antibody.

2. "Nested" combinatorial library protocols

In this type of protocol libraries of sublibraries are screened, and a sublibrary selected for further screening [see, e.g., Zuckermann et al. (1994) J. Med. Chem. 37:2678-2685; and Zuckermann et al. (1992) J. Am. Chem. Soc. 114:10646-10647]. In this method, three sets of monomers were

chosen from commercially available monomers, a set of four aromatic hydrophobic monomers, a set of three hydroxylic monomers, a set of seventeen diverse monomers, and three N-termini were selected. The selection was based on an analysis of the target receptor and known ligands. A library containing eighteen mixtures, generated from the six permutations of the three monomer sets, times three N-termini was prepared. Each mixture of all combinations of the three sets of amines, four sets of hydrophobic monomers and seventeen diverse monomers was then assayed. The most potent mixture was selected for deconvolution by synthesis of pools of combinatorial mixtures of the components of the selected pool. This process was repeated, until individual compounds were selected.

Tagging the mixtures with the matrices with memories will greatly simplify the above protocol. Instead of screening each mixture separately, each matrix particle with memory will be prepared with sets of the compounds, analogous to the mixtures of compounds. The resulting matrix particles with memories and linked compounds can be combined and then assayed. As with any of the methods provided herein, the linked compounds [molecules or biological particles] can be cleaved from the matrix with memory prior to assaying or anytime thereafter, as long as the cleaved molecules remain in proximity to the device or in some manner can be identified as the molecules or particles that were linked to the device. The matrix particle(s) with memories that exhibit the highest affinity [bind the greatest amount of sample at equilibrium] are selected and identified by querying the memory to identify the group of compounds. This group of compounds is then deconvoluted and further screened by repeating this process, on or off the matrices with memories, until high affinity compounds are selected.

3. Other combinatorial protocols

The matrices with memories provided herein may be used as supports in any synthetic scheme and for any protocol, including protocols for synthesis of solid state materials. Combinatorial approaches have been developed for parallel synthesis of libraries of solid state materials [see, e.g., Xiang et al. (1995) Science 268:1738-1740]. In particular, arrays containing different combinations, stoichiometries, and deposition sequences of inorganics, such as $BaCO_3$, BiO_3, CaO, CuO, PbO, $SrCO_3$ and Y_2O_3, for screening as superconductors have been prepared. These arrays may be combined with memories that identify position and the array and/or deposited material.

IV. EXAMPLES

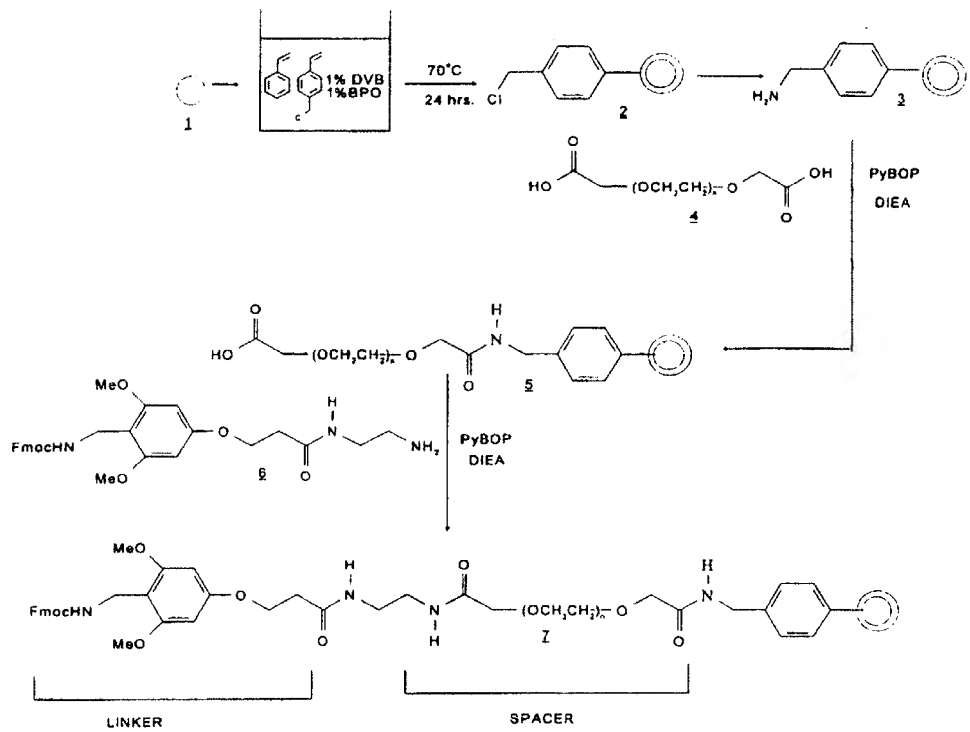
The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

EXAMPLE 1

Formulation of a polystyrene polymer on glass and derivatization of polystyrene

A glass surface of any conformation [beads for exemplification purposes (1)] that contains a selected memory device that coats the device or that can be used in proximity to the device or subsequently linked to the device is coated with a layer of polystyrene that is derivatized so that it contains a cleavable linker, such as an acid cleavable linker. To effect such coating a bead, for example, is coated with a layer of a solution of styrene, chloromethylated styrene, divinyl benzene, benzoyl peroxide [88/10/1/1/, molar ratio] and heated at 70° C for 24 h. The result is a cross-linked chloromethylated polystyrene on glass (2). Treatment of (2) with ammonia [2 M in 1,4-dioxane, overnight] produces aminomethylated coated beads (3). The amino group on (3) is coupled with polyethylene glycol dicarboxymethyl ether (4) [$n \approx 20$] under standard conditions [PyBop/DIEA] to yield carboxylic acid derivatized beads (5). Coupling of (5) with modified PAL [PAL is

pyridylalanine] linker (6) under the same conditions produces a bead that is coated with polystyrene that has an acid cleavable linker (7).



The resulting coated beads with memories are then used as solid support for molecular syntheses or for linkage of any desired substrate.

EXAMPLE 2

Construction of a matrix with memory

A matrix with memory was constructed from (a) and (b) as follows:

(a) A small (8 x 1 x 1 mm) semiconductor memory device [the IPTT-100 purchased from Bio Medic Data Systems, Inc., Maywood, NJ; see, also U.S. Patent Nos. 5,422,636, 5,420,579, 5,262,772, 5,252,962, 5,250,962, 5,074,318, and RE 34,936].

The memory device is a transponder [IPTT-100, Bio Medic Data Systems, Inc., Maywood, NJ] that includes a remotely addressable memory [EEPROM]. The transponder receives, stores and emits radio frequency signals of different frequencies so that it can be remotely programmed with information regarding synthetic steps and the constituents of linked or proximate molecules or biological particles. These devices are designed to operate without a battery, relying on the energy generated by the radio frequency pulses used in the encoding process. Also, it is important to note that additional sensors such as temperature [as in this case], pH, or concentration measuring devices can be installed. The resulting combinations are capable of withstanding most reagents and conditions used in synthetic organic chemistry, including temperatures from -78 to 150° C.

The transponder was encoded and read with a device that emits and reads RF frequencies [Bio Medic Data Systems Inc. DAS-5001 CONSOLE™ System, see, also U.S. Patent No. 5,252,962].

These memory devices include EEPROM (Electrical, Erasable, Programmable, Read-Only Memory) "flash" unit and a temperature sensing device able to accept or emit information at any time. At each step of the combinatorial "split and pool" sequence, encoding information is sent from a distance in the form of radio frequency pulses at 145 kHz and stored until decoding is needed. When needed the radio frequency signals are retrieved using a specially assembled apparatus capable of reading the radio frequency code from a distance [DAS-5001 CONSOLE™ from Bio Medic Data Systems, Inc., Maywood, NJ; see, e.g., U.S. Patent Nos. 5,422,636, 5,420,579, 5,262,772, 5,252,962 and 5,250,962, 5,252,962 and 5,262,772].

(b) TENTAGEL™ polymer beads carrying an acid-cleavable linker [TENTAGEL S Am cat # S30 022, RAPP Polymer, Tubingen, Germany].

(c) A chemically inert surrounding porous support [polypropylene AA, SPECTRUM, Houston, TX].

One transponder and about 20 mg of the derivatized TENTAGEL™ beads have been sealed in a small [of a size just sufficient to hold the beads and transponder] porous polypropylene microvessel [see, Examples 3 and 4].

EXAMPLE 3

MICROVESSELS

A. FIGURES 11-13

FIGURES 11-13 illustrate an embodiment of a microvessel 20 provided herein. The microvessel 20 is a generally elongated body with walls 22 of porous or semi-permeable non-reactive material which are sealed at both ends with one or more solid-material cap assemblies 42, 44. The microvessel 20 retains particulate matrix materials 40 and one or more recording devices 34. In the preferred embodiment illustrated in FIGURES 11-13, the recording device includes a shell 36 that is impervious to the processing steps or solutions with which the microvessel may come into contact, but which permits transmission of electromagnetic signals, including radiofrequency, magnetic or optical signals, to and from the recording media of the recording device.

The preferred microvessel 20 is generally cylindrically shaped and has two solid-material cap assemblies 42, 44. The cap assemblies may be formed of any material that is non-reactive with the solutions with which the microvessel will come into contact. Such appropriate materials include, for example, plastic, TEFLON, polytetrafluoroethylene (hereinafter, PTFE) or polypropylene. Each cap assembly 42, 44 preferably includes a support base 26, 28, respectively, and an end cap 24, 30, respectively. Each support base 26, 28 is permanently attached to the walls 22 of the vessel by known means such as bonding with appropriate adhesives or heat treatment, either by heat-shrinking the wall material onto the lower portions of the support bases 26,28, or by fusing the wall material with the support base material.

Preferably, at least one of the caps 24,30 is removably attached to its cap base 26, for example by providing complementary threads on the support base and the end cap so that the end cap can be screwed into the support base, as illustrated in Figure 12. Other possible means for attaching the end cap to the support base will be apparent to those in the art, and can include snap rings, spring tabs, and bayonet connectors, among others. The end cap 24, has one or more slots, bores, or recesses 32 formed in its outer surface to facilitate removal or replacement, with the user's fingers and/or by use of an appropriate tool. For the example illustrated, a spanner wrench having pegs spaced at the same separation as the recesses 32 can be used by inserting the pegs into the recesses. For a single slot, removal and replacement of the end cap could be achieved by using a screwdriver. Protruding tabs, rims, knurled edges or other means to enhance the ability to grasp the end cap can be used for manual assembly/disassembly of the microvessel. The cap assembly 42 at the opposite end of the microvessel can be permanently sealed using an adhesive or heat treatment to attach the support base 28 to the end cap 30, or the cap assembly 42 can be molded as a single piece, combining the support base 28 and the end cap 30.

Retained within the microvessel 20 are particle matrix materials 40 and a memory device 34. The recording device 34, in the preferred embodiment illustrated, includes a data storage unit(s) 38 and a shell 36 that protects the recording device 38 from the processing steps and/or solutions to which the microvessel are subjected. This shell 36 is preferably constructed of material that is non-reactive with and impervious to the solutions with which the microvessel may come into contact, and which is penetrable by the electromagnetic radiation, or similar means, used to read from and write to the memory device. One presently preferred device is a modified form of the IPTT-100 [Bio Medic Data Systems, Inc. ("BMDS"), Maywood, NJ; see, also U.S. Patent Nos. 5,422,636, 5,420,579, 5,262,772, 5,252,962 and 5,250,962], which generally contains an electrically programmable memory chip 130 and decoding and power

conversion circuitry 132 mounted on an elongated ceramic circuit board 134 and connected to an LC oscillator, comprising capacitor 138 and coil 136 wound around a ferrite core, which inductively receives and responds to a frequency-modulated magnetic signal generated by a similar LC oscillator in the write device, allowing the device to be remotely encoded and remotely read at a distance on the order of 1 cm or less. The device has been modified from the supplier's standard commercially-available form to provide physical dimensions to facilitate placement in the microvessel 20. The modification involves application of the simple and well-known relationship between inductor core area and length, the permeability of the core material, and the number of windings, i.e., L (inductance) $= N^2\mu A/l$, where N is the number of windings, μ the permeability of the core, A is the core area and l is the core length.

Other remotely programmable and readable tags are commercially available which may be used in the inventive system, such as those manufactured by Identification Device Technology, UK, which are also presently preferred. These devices have circuitry and operational parameters similar to the device described above, but it may be necessary to modify the coil to reduce the access range to less than or equal to 1 cm. It is generally preferred that the responder, i.e., the memory device, and the transceiver in the control system be from the same manufacturer to assure complete compatibility.

The illustrated microvessel, as illustrated in FIGS. 11-13, is of a size sufficient to contain at least one recording device and one matrix particle, such as a TENTAGEL™ bead. The device is typically 20 mm in length [i.e., the largest dimension] or smaller, with a diameter of approximately 5 mm or less, although other sizes are also contemplated. These sizes are sufficient to contain form about 1 mg up to about 1 g of matrix particle, and thus range from about 1 mm up 100 mm in the largest dimension, typically about 5 mm to about 50 mm, preferably 10 mm to 30 mm, and most preferably about 15 to 25 mm. The size, of course can be smaller than those specified

or larger. The wall material of the microvessel is PTFE mesh having a preferably about 50 μM to 100 μM, generally 50 to 70 μM hole size that is commercially available. The size of course is selected to be sufficiently small to retain the matrix particles. The cap apparatus is machined rod PTFE [commercially available from McMaster Carr, as Part #8546K11].

The matrix material is selected based upon the particular use of the microvessel; for example, a functionalized resin, such as TENTAGEL™ resin, commercially available from Rapp Polymere, Tubingen, Germany, is preferred for use in peptide synthesis and similar processes. The matrix material may also include fluophores or scintillants as described herein.

Alternative embodiments of the microvessel will be appreciated and include, for example, a pouch, including porous or semi-permeable material, which is permanently sealed to itself and contains matrix material and one or more memories.

B. FIGURES 14-16

FIGURES 14-16 illustrate an alternate embodiment of a microvessel provided herein. Like the microvessel described in Example 3, this embodiment of the microvessel also retains particulate matrix materials and one or more recording devices (not illustrated). The microvessel has a single-piece solid material frame 82, including a top ring 84, two support ribs 88, 100 disposed diametrically opposite each other and a bottom cap 86. The solid material frame 82 may be constructed of any material which is non-reactive with the solutions with which the microvessel will come into contact. Such appropriate materials include, for example, plastic, polytetrafluoroethylene (hereinafter, PTFE), TEFLON or polypropylene, and formation may be by molding or machining of the selected material, with the former being preferred for economy of manufacture.

The sidewall of the microvessel 98 is formed of porous or semi-permeable non-reactive material, such as PTFE mesh, preferably having a 70μM pore size. The sidewall is preferably attached to the top ring 84 and bottom cap 86 of the solid material frame 82. Such attachment may be by

known means such as bonding with appropriate glues or other chemicals or heat, with heat being preferred.

In the embodiment of FIGURES 14-16, the two support ribs 88, 100 are positioned opposite one another, however, any number of support ribs, i.e., one or more, may be provided. The microvessel sidewall 98 need not be fully attached to the support ribs 88, 100, however, the molding process by which the microvessels are formed may result in attachment at all contact points between the frame and the sidewall.

In the preferred manufacturing process, the sidewall material, a flat sheet of mesh, is rolled into a cylinder and placed inside the mold. The frame material is injected into the mold around the mesh, causing the frame to fuse to the mesh at all contact points, and sealing the edges of the mesh to close the cylinder.

In the embodiment illustrated in FIGS. 14-15, the microvessel is configured with a removable end cap 90. The end cap 90 is preferably constructed of the same material as the solid material frame 82. A snap ring, or, as illustrated, projections 92, 94 extend downward from the inside surface of the end cap 90. The projections 92, 94 have a flange which mates with a groove 96 formed in the inner wall of top ring 84 when pressed into the top ring to releasable secure the end cap 90 to the microvessel 80. As will be apparent, other means for releasably securing the end cap 90 to the top ring 84 can be used, including, but not limited to, those alternatives stated for the embodiment of FIGURES 11-13. The dimensions vary as described for the microvessel of FIGURES 11-13 and elsewhere herein.

In other embodiments, these vessels fabricated in any desired or convenient geometry, such as conical shapes. They can be solid at one end, and only require a single cap or sealable end.

These microvessels are prefereably fabricated as follows. The solid portions, such as the solid cap and body, are fabricated from a polypropylene resin, Moplen resin (e.g., V29G PP resin from Montell,

Newark DE, a distributor for Himont, Italy]. The mesh portion is fabricated from a polypropylene, polyester, polyethylene or fluorphore-containing mesh [e.g., PROPYLTEX®, FLUORTEX®, and other such meshes, including cat. no. 9-70/22 available from TETKO* Inc, Briarcliff Manor, NY, which prepares woven screening media, polypropylene mesh, ETF mesh, PTFE mesh, polymers from W.L. Gore. The pores are any suitable size [typically about 50-100 μM, depending upon the size of the particulate matrix material] that permits contact with the synthetic components in the medium, but retains the particulate matrix particles.

EXAMPLE 4

Manual system

Illustrated in FIGURE 17 is a program/read station for writing to and reading from the memory devices in the microvessel. The electronic components are commercially available from the same supplier of the memory devices, e.g., BMDS or ID TAG [Bracknell Berks RG12 3XQ, UK], so that the basic operations and frequency are compatible. The basic controller 170 and the transceiver 172 are disposed within a housing 174 which has a recessed area 176 positioned within the transmission range of coil 178. The microvessel 180 may be placed anywhere within recessed area 176, in any orientation, for both programming and reading functions. Basic controller 170 is connected to the system controller 182, illustrated here as a functional block, which provides the commands and encoded data for writing to the memory device in the microvessel and which receives and decodes data from the memory device during the read function. System controller 182 is typically a PC or lap top computer which has been programmed with control software 184 for the various write and read functions.

An example of the operation of the system of FIGURE 17 is illustrated in FIGURE 18. When power is supplied to the system, transceiver 172 emits an interrogation signal 185 to test for the presence of a memory device, i.e., a responder, within its detection range. The interrogation signal

185 is essentially a read signal that is continuously transmitted until a response 186 is received. The user manually places a microvessel 180 within the recessed area 176 so that the interrogation signal 185 provides a response to the controllers indicating the presence on the microvessel. The system receives the interrogation signal and performs a decode operation 187 to determine the data on the memory device within the microvessel, which data may include identification of the device and data concerning prior operations to which the microvessel has been exposed. Based upon the data obtained, the system makes a determination 188 of whether additional information is to be written. The system then performs a write operation 189 to record the immediately preceding operation. The write operation 189 involves modulating the transmitted signal as a series of "0's" and "1's", which are recorded on the memory chip, which typically has a 128 bit capacity. After completion of the programming step 189, an error check 190 is performed wherein a second read signal is emitted to verify the data that was written for integrity and correct content. If the correct data is not verified, the system may attempt to perform the write operation 189 again. After verification of the correct data, if the microvessel is one that should proceed to another operation, the system controller 182 will display instructions 192 for direction of the microvessel to the next process step.

The read operation is the same as the beginning of the write operation, with the interrogation signal being continuously transmitted, or transmitted at regular intervals, until a response is received. The response signal from the memory device in the microvessel 180 is conducted to system controller 182 for decoding and output of the data that is stored on the memory device. Software within the system controller 182 includes a data base mapping function which provides an index for identifying the process step associated with data written at one or more locations in the memory device. The system memory within the system controller 182 will retain the identification and process steps for each microvessel, and an output display of the information relating to each microvessel can indicate

both where the microvessel has been, and where it should go in subsequent steps, if any. After the data stored within the microvessel has been read, it is removed from the interrogation field and advanced to its next process step.

Software for aiding in the steps in combinatorial synthesis schemes has been developed. The software, which in light of the description herein can be written, facilitates the process of creating chemical libraries with the systems provided herein. The exemplied software, now available under the name ACCUTAG™ SYNTHESIS MANAGER Software as an integral part of the AccuTag™-100 Combinatorial Chemistry System [e.g., an embodiment of the system provided herein]. These systems exemplified with the device of Figure 17 [e.g., sold under the name ACCUTAG™], computer-based hardware, and the matrix with memories used therewith, such as the MICROKAN matrix with memory device and the MICROTUBE matrix with memory device [see, e.g., Figures 11-15 and 21].

The SYNTHESIS MANAGER software is organized into the following sections. These sections represent the normal sequence of activities that go into building a library with the system provided herein.

1. Define Building Blocks. The user enters the names of the chemical building blocks to be used. For brevity of reference, a code letter is assigned to each building block.
2. Plan Steps.
    a. Number of Steps. The user specifies the number of steps. In a given step, a building block, such as a monomer, amino acid, nucleotide, will be chemically added to each compound that is being synthesized.
    b. Building Blocks To Use. The user specifies which of the defined building blocks will be used in each step.

    If, for example, there are 3 steps and the user specifies building blocks A, B, C in step 1, building blocks D, and number in step 2, and building blocks F, G, H, I in step 3, then

the resulting library will contain 24 unique compounds because there are 3 x 2 x 4 = 24 combinations of building blocks.

c. Procedural information. The user optionally enters "recipe" information such as reaction times, temperatures, molarities, and reagents to use for each building block's reactions as well as procedures common to all building blocks. At the appropriate times during the "Perform Synthesis" section of the program, this information is "played back" to the user. This is a convenience function for the user.

3. Perform Synthesis. Using a virtual library database of all the involved building blocks, reactions, process and compound tracking data, the software facilitates the step-by-step synthesis of the chemical library using memories with matrices, such as a MICROKAN OR MICROTUBE. For each step specified in Plan Steps (above) the following four tasks are performed.

a. Pre-Procedure. Any preliminary procedures that the user entered are displayed. Typically these will involve chemical "deprotection" of the reaction site associated with this step.

b. Sorting. The "directed sorting" process for the current step is administered by the software. The user is prompted to place a memory with matrix on the scanning station [see, e.g., Figure 17], which is connected to a computer. The memory in the matrix, i.e., the tags, identification [ID] is read. The software does a database look up, seeking this unique ID.

On the first step, the tag's ID is not found in the data base, so the software assigns it to the first compound in the library, which has not yet been associated with a tag. The user is instructed to place the device into the reaction vessel for the appropriate building block. From this point on, when this tag is read, the user is instructed to put the device into the reaction

that will add the building block planned for this step for this specific compound.

c. Reactions. Through directed sorting, all the devices in the library are now in reaction vessels. These is one vessel for each building block in the current step. The user is now prompted to perform the synthetic chemistry that will add each vessel's building block to the compounds it contains. The software displays any procedure information pertaining to reaction conditions that the user entered in Plan Steps.

d. Work Up. The user is prompted to perform the "work up" [follow-up] task. Any work-up procedures the user entered in Plan Steps are displayed. Typically these involve rinsing and drying the reactor devices.

4. Archive. Archive refers to the process of transferring the completely synthesized compound from matrices with memories to a storage medium, such as a 96 well microplate or vials of any shape or size. This works as follows.

a. User chooses either vials or microplates [or other containier]. These containers or vials may include memories into which identifying information can be entered, such as by scanning the first memory and then entering the scanned information into the memory in the matrix [container] into which the compounds are transferred.

b. User places device on memory with matrix reader, a scanning station [see, e.g., Figure 17].

c. User selects a placement location: a well in a plate or a specific vial number.

d. User affirms placement location and the database is updated to document this. Chemically, the user typically cleaves the compound from the solid phase support and deposits only the

synthesized compound in the storage media, while salvaging the reusable tag device for reuse on a another library.

e. The software automatically selects the next storage location. The user may override this, and make another selection.

While not required part of the process, additional functions, such as the following functions are provided.

1. Utility Functions.
   a. Decode Tags. Using this function, at any time, the user can place a tag on the Scanning Station. If the tag has been assigned to a compound in the library, then information about that compound is displayed.
   b. Find Compound. The user can specify a combination of building blocks. The software looks up this combination, and if it exists, it displays information about the compound and its tag.
   c. Status. Spreadsheets showing all devices, their building block assignments and process status (which steps have been sorted) is shown.
2. Printing. The user can print out report describing:
   a. Building Blocks
   b. Steps planned.
   c. List of All Compounds.
3. On Line Help. The user can get context-sensitive assistance and a hypertext version of the System's User's Guide.

EXAMPLE 5

Preparation of 432 member libraries

The following Example describes synthesis set forth in FIGURE 36. Libraries using the building block sets set forth in FIGURES 37 and 38 were prepared. All reagents were obtained from Aldrich, except for the Knorr linker and PyBOP, which were obtained from from Novabiochem. In the

protocol depicted in FIGURE 36 using the building blocks in FIGURES 37 or 38, Knorr linkers were used. Steps below refer to the letters in scheme II set forth in FIGURE 36.

Using solid-phase organic synthesis, two 432-member (18x18x3) libraries based on the AG490 tyrphostin template. By using 432 microreactors each equipped with a unique radiofrequency memory ID tag, the 432 products could be obtained as discrete entities (i.e., not as mixtures) via only 18+8+3, or 29 reactions. Reading each ID tag after each reaction step permitted the directed sorting of reactors into appropriate reaction vessels containing multiple reactors. After synthesis, all products were cleaved from the solid-phase support and lyophilized to afford powders. Rigorous analysis of 5% of the library members for one of the libraries (FIGURE 37), in addition to TLC analysis of every library member, demonstrated that each sample is a single (>85%of homogeneous) compound. Some 88% of these compounds were obtained in amounts of between 5 and 19 mg. Using this reaction sequence and the directed sorting approach, the synthesis of much larger AG490-based libraries can be obtained.

Encoding MICROTUBE® microreactors with RF

The identity of twenty-nine building blocks were entered into the data base and used with ACCUTAG SYNTHESIS MANAGER software. The eighteen building blocks BB1 were used for the reductive alkylation in the first step, eight for the Aldol condensation in the second step and three in the esterification in the last step in the synthesis of tyrphostin. The 432 MICROTUBE® microreactors, containing the IDTAG tag, for the library were encoded with radio freguency and their synthetic histories in were recorded during synthesis.

a) **Addition of linkers to the MICROTUBE® microreactors**

To 445 MICROTUBE® microreactors in 400 ml of methylene chloride in a 1 L jar with a teflon cap was added sequentially 28.4g (52.5 mmol) of Knorr linker, 3.5g (105 mmol) of DIEA, and 28.6g (55 mmol) of PyBOP.

The mixture was put on an orbital shaker at room temperature overnight. After the solution was removed by an aspiration, the MICROTUBE® microreactors were washed two times sequentially with DMF, DCM, and MeOH over 20 min on a shaker. The MICROTUBE® microreactors were dried in vacuum over 24 hours after washed with ethyl ether.

b) Capping MICROTUBE® microreactors

The above dry MICROTUBE® microreactors were treated with 400 ml of solution of acetic anhydrous (0.5 M) and DIEA (0.6 M) in methylene chloride for 1.5h. After the solution was removed by an aspiration, the MICROTUBE® microreactors were washed two times sequentially with DMF, DCM, and MeOH over 20 min on a shaker. The MICROTUBE® microreactors were dried in vacuum over 24 hours after washed with ethyl ether. Kaiser test showed negative.

c) Measurement of Loading

The above 445 MICROTUBE® microreactors were treated with 450 ml of 20% piperidine in DMF for 1 h. The loading of the MICROTUBE® microreactors was measured by UV absorption of solution at 301nm. The loading in average is 52.7 mmol per MICROTUBE® microreactor. After the solution was removed by an aspiration, the MICROTUBE® microreactors were washed two times sequentially with DMF, DCM, and MeOH over 20 min on a shaker. The MICROTUBE® microreactors were dried in vacuum over 24 hours after washed with ethyl ether.

d) and e) Reductive Alkylation

The MICROTUBE® microreactors were sorted into 18 bottles according the directions determined by ACCUTAG SYNTHESIS MANAGER. Each bottle containing 24 MICROTUBE® microreactors was treated with 25 mL of trimethyl orthoformate. Then, an aldehyde (R^1CHO, 12.5 mmol) from the building blocks for the reductive alkylation was added to the corresponding bottle and the resulting mixture was shaken on a orbital shaker for 3 h. After 1.26g (20 mmol) of $NaCNBH_3$ was added to each bottle for 20 min, 0.5 mL of acetic acid was added to each bottle and shaking was continued

for 3h. After the solvents were removed by an aspiration, MICROTUBE® microreactors in each bottle were washed quickly with MeOH one time. The MICROTUBE® microreactors were combined in 1 L jar and washed two times sequentially with DMF, DCM, and MeOH during a 20 min period on a shaker. The MICROTUBE® microreactors were dried in vacuum for 24 hours after being washed with ethyl ether.

f) Cyanoacetylation

The combined MICROTUBE® microreactors in the 1 L jar were treated with DIEA (51.6g, 400 mmol), cyanoacetic acid (17g, 200 mmol) and diisopropylcarbondiimide (32.8g, 260 mmol) in 400 ml of anhydrous DMF overnight. After the solvent was removed, the MICROTUBE® microreactors were washed with 200 ml of anhydrous DMF two times the above coupling was repeated two times. After the solution was removed by aspiration, the MICROTUBE® microreactors were washed two times sequentially with DMF, DCM, and MeOH during a 20 min period on a shaker. The MICROTUBE® microreactors were dried in vacuum for 24 hours after being washed with ethyl ether.

g) **Aldol condensation**

The MICROTUBE® microreactors were sorted into 8 bottles according to the distribution determined by with the ACCUTAG SYNTHESIS MANAGER software. Each bottle, which contained 54 MICROTUBE® microreactors was treated with 50 ml anhydrous DMF, 5 ml MeOH, and piperidine (850mg, 10 mmol). Then an ahdehyde ($OHC\text{-}R^2\text{-}(OH)_2$, 27.5 mmol) from the building blocks for the Aldol condensation was added to each corresponding bottle and the resulting mixtures were shaken for two days. After the solvents were removed by aspiration, the MICROTUBE® microreactors in each bottle were washed quickly with DMF one time. The MICROTUBE® microreactors were combined in a 1 L and were washed two times sequentially with DMF, DCM, and MeOH during a 20 min period on a shaker. The MICROTUBE® microreactors were dried in vacuum for 24 hours after being washed with ethyl ether.

h) Esterification

The MICROTUBE® microreactors were sorted into 3 bottles as directed by the ACCUTAG SYNTHESIS MANAGER software. The first one of bottles was treated with nothing, the second with triethylamine (15g, 150 mmol) and acetic chloride (5.89g, 75 mmol) in DCM, and the third with triethylamine (15g, 150 mmol) and benzoic chloride (10.5g, 75 mmol) in anhydrous DMF overnight. After the solvents were removed by aspiration, the MICROTUBE® microreactors in each bottle were washed two times sequentially with DMF, DCM, and MeOH during a 20 min period on a shaker. The MICROTUBE® microreactors were dried in vacuum for 24 hours after being washed with ethyl ether.

i) Cleavage

Each MICROTUBE® microreactor, which contained linked tyrphostins, was sorted into a vial as directed by the ACCUTAG SYNTHESIS MANAGER software. The tyrphostins with free hydroxy groups were treated with 4% TFA in dioxane (3.5 ml per MICROTUBE® microreactor) and the tyrphostins without a free hydroxy group were treated with 4% TFA in benzene (3.5 ml per MICROTUBE® microreactor) for 1 h on a shaker. All products were determined with TLC and one clear spot for each solution was shown on the plate. After the MICROTUBE® microreactors were removed from the vials and washed in dioxane or benzene, the solutions in vials were frozen and then lyophilized.

The products were obtained in average 10 mg per MICROTUBE® microreactor. To verify the accuracy of the reading and writing and synthesis protocol, rndomly, 24 products of the library were determined by NMR, MS and HPLC. All these NMR spectra were correct and clear. All MS gave the correct molecular weights. The purity of 24 products in HPLC (254nm) ranged from 56-100% (> 90% 5 products, 80-90% 4 products, 70-80% 13 products, 66% 1 products, 57% 1 products). The purity is artificially low because the corresponding compounds have low absorption at 254 nm. If absorption had been measured in the maximum absorption

peaks, their purity percentages would be much higher. Since the NMR spectra were clear and correct, the products must be very pure.

EXAMPLE 6

Preparation of ~10K member libraries

The above procedure is repeated except that the building blocks set forth in FIGURES 39 A-C are used in place of those set forth in FIGURES 37 or 38. The number of microreactors is increased to about 13 K. The R group in FIGURE 39B is any group, and is preferably any of the groups set forth for R^4 or any of the other substituents set forth for R^2.

Encoding MICROTUBE® microreactors with RF

The identity of eighty-six building blocks are entered into the data base and used with ACCUTAG SYNTHESIS MANAGER software. The fifty building blocks BB1 are used for the reductive alkylation in the first step, twenty-six for the Aldol condensation in the second step and ten in the esterification in the last step in the synthesis of the tyrphostin analogs. The 13,000 MICROTUBE® microreactors for the library are encoded with radio freguency and their synthetic histories are recorded during the synthesis. Amounts of materials and time for each step and reaciton are determined by reference to the previous EXAMPLE and depend upon the number and size of the microreactors used.

a) Addition of linkers to the MICROTUBE® microreactors

To the 13K MICROTUBE® microreactors in methylene chloride in a suitable container are sequentially added appropriate amounts of the Knorr linker, DIEA PyBOP. The mixture is put shaken at room temperature overnight. After the solution is removed, the MICROTUBE® microreactors are washed two times sequentially with DMF, DCM, and MeOH while being shaken. The MICROTUBE® microreactors are dried in vacuum for about 24 hours after being washed with ethyl ether.

b) Capping MICROTUBE® microreactors

The above dry MICROTUBE® microreactors are treated with a solution of acetic anhydrous and DIEA in methylene chloride. After the solution is

removed the MICROTUBE® microreactors are washed two times sequentially with DMF, DCM, and MeOH while being shaken. The MICROTUBE® microreactors are dried in vacuum for about 24 hours after being washed with ethyl ether.

c) **Measurement of Loading**

The above MICROTUBE® microreactors are treated as described in the previous EXAMPLE with 20% piperidine in DMF. The loading of the MICROTUBE® microreactors is measured by UV absorption of solution at 301 nm. After the solution is removed by an aspiration, the MICROTUBE® microreactors are washed two times sequentially with DMF, DCM, and MeOH while being shaken. The MICROTUBE® microreactors are dried in vacuum for about 24 hours after being washed with ethyl ether.

d) and e) Reductive Alkylation

The MICROTUBE® microreactors are sorted into 50 suitable containers according to the directions determined by ACCUTAG SYNTHESIS MANAGER software. Each bottle containing about 260 MICROTUBE® microreactors is treated with trimethyl orthoformate. Then, an aldehyde (R^1CHO) from the building blocks for the reductive alkylation is added to the corresponding bottle and the resulting mixture is shaken for about 3 h. After an appropriate amount (determined by from the previous EXAMPLE) of $NaCNBH_3$ is added to each bottle, acetic acid is added to each bottle and shaking is continued for about 3 h. After the solvents are removed by an aspiration, MICROTUBE® microreactors in each bottle are washed quickly with MeOH one time. The MICROTUBE® microreactors are combined in a suitable container and washed two times sequentially with DMF, DCM, and MeOH during a suitable period while being shaken. The MICROTUBE® microreactors are dried in vacuum for about 24 hours after being washed with ethyl ether.

f) Cyanoacetylation

The combined MICROTUBE® microreactors in the container are treated with DIEA, cyanoacetic acid and diisopropylcarbondiimide in of anhydrous DMF overnight. After the solvent is removed, the MICROTUBE® microreactors are washed with anhydrous DMF two times the above coupling is repeated two times. After the solution is removed, the MICROTUBE® microreactors are washed two times sequentially with DMF, DCM, and MeOH while being shaken. The MICROTUBE® microreactors are dried in vacuum for about 24 hours after being washed with ethyl ether.

g) Aldol condensation

The MICROTUBE® microreactors are sorted into 26 container according to the distribution determined by with the ACCUTAG SYNTHESIS MANAGER software. Each bottle, which contains 500 MICROTUBE® microreactors are treated with anhydrous, 5 ml MeOH, and piperidine. Then an ahdehyde (OHC-R^2-$(OH)_2$ from the building blocks (BB2) for the Aldol condensation is added to each corresponding container and the resulting mixtures are shaken for about two days. After the solvents are removed by aspiration, the MICROTUBE® microreactors in each bottle are washed quickly with DMF one time. The MICROTUBE® microreactors are combined into a single vessel and are washed two times sequentially with DMF, DCM, and MeOH while being shaken. The MICROTUBE® microreactors are dried in vacuum for about 24 hours after being washed with ethyl ether.

h) Esterification

The MICROTUBE® microreactors are sorted into 10 containers as directed by the ACCUTAG SYNTHESIS MANAGER software and treated with the reagents set forth as BB3 (R^3-X). After the solvents are removed by aspiration, the MICROTUBE® microreactors in each container are washed two times sequentially with DMF, DCM, and MeOH while being shaken. The MICROTUBE® microreactors are dried in vacuum for about 24 hours after being washed with ethyl ether.

i) Cleavage

Each MICROTUBE® microreactor, which contained linked tyrphostins, was sorted into a vial as directed by the ACCUTAG SYNTHESIS MANAGER software. The tyrphostins with free hydroxy groups are treated with 4% TFA in dioxane and the tyrphostins without a free hydroxy group are treated with 4% TFA in benzene for 1 h on a shaker. After the MICROTUBE® microreactors are removed from the vials and washed in dioxane or benzene, the solutions in vials are frozen and then lyophilized.

Alternatively, the tyrphostins may be stored on the microreactor and used when needed. Portions of the microreactor may be chopped off and the linked compounds subjected to analysis and/or screening as needed.

EXAMPLE 7

Preparation of the compounds designated d6Bz

With reference to the building blocks in Figure 37, a single compound designed d6Bz was prepared as outlined in scheme II, FIGURE 36. The linking and capping steps were performed as in the previous EXAMPLE.

d) and e) Reductive Alkylation

One MICROTUBE® microreactor (known loading) with deprotected Knorr linkern was treated with 2 ml of 0.25M 4-florobenzaldehyde in trimethyl orthoformate in a glass vial with a Teflon cap on a shaker for 2h. Then, 63mg (1 mmol) of $NaCNBH_3$ was added. After 10min., 40ml of acetic acid was added and shaking was continued for 2 h. After the solvent was removed by aspiration, the MICROTUBE® microreactor was washed two times sequentially with DMF, DCM, and MeOH over 5 min on a shaker. The MICROTUBE® microreactor was dried in vacuum for 4 hours.

f) Cyanoacetylation

The MICROTUBE® microreactor was treated with DIEA (258mg, 2mmol), cyanoacetic acid (85mg, 1mmol), and diisopropylcarbondiimide (164mg, 1.3mmol) in 2 ml of anhydrous DMF overnight. After the solvent was removed, the MICROTUBE® microreactor was washed with anhydrous DMF two times and the above coupling was repeated. After the solution was removed by aspiration, the MICROTUBE® microreactor was washed

two times sequentially with DMF, DCM, and MeOH during a 5 min period on a shaker. The MICROTUBE® microreactor was dried in vacuum for 4 hours.

g) Aldol condensation

The MICROTUBE® microreactor was treated with 2 ml of anhydrous DMF, 0.2 ml of MeOH, 3-methyl-4-hydroxy-benzaldehyde (68mg, 0.5mmol) and piperidine (21mg, 0.25mmol) for two days on a shaker. After the solvent was removed by aspiration, and the microreactor was washed two times sequentially with DMF, DCM, and MeOH during a 5 min period on a shaker. The MICROTUBE® microreactor was dried in vacuum for 4 hours.

h) Esterification

The MICROTUBE® microreactor was treated with triethylamine (202mg, 2mmol)) and benzoic chloride (140.5mg, 1mmol) in 2 ml of anhydrous DMF overnight. After the solvent was removed by aspiration, the MICROTUBE® microreactor was washed two times sequentially with DMF, DCM, and MeOH during a 20 min period on a shaker. The MICROTUBE® microreactor was dried in vacuum for 4 hours.

i) Cleavage

The MICROTUBE® microreactor was treated with 2 ml of 2% TFA in methylene chloride in a vial with a Teflon cap for 1 h on a shaker. The MICROTUBE® microreactor was removed from the vial and washed. The solution in the vial was removed by rotavap and the residue was dried in vacuum. The product, usually obtained in 80-90% yield, is pure enough for good NMR and MS spectra. NMR ($CDCl_3$, 500MHz): d2.31 (s, 3H), d4.58 (d, J =4, 2H), d6.70 (t, J =4, 1H), d7.06 (t, 2H), d7.34 (m, 2H), d7.55 (m, 3H), d7.71(t, 1H), d8.25 (d,2H), d8.35 (s,1H). MS (electrospray): MH^+ = 415, MNa^+ = 437, $[M-H]^-$=413, $M+Cl^-$ = 449.

EXAMPLE 8

ASSAYS FOR EVALUATING THE CELL GROWTH AND PTK INHIBITORY ACTIVITY OF COMPOUNDS

The libraries provided herein contain compounds that modulate the activity of tyrosine kinases. Identification of the compounds from the library

requires use of an assay for the desired activity. Any assay known to those of skill in the art that identifies compounds that modulate (i.e., alter) the activity of a tyrosine kinase may be used. The preferred assay is that provided herein. Compounds in the libraries provided herein have been tested and shown to have activity in this assay. Such compounds are candidates for further investigation as tyrosine kinase inhibitors, and, hence as pharmaceuticals for treatment of disorders, particularly proliferatie disorders, including cancers, particularly leukemias, in which tyrosine kinase activity has a role. Thus, compounds provided herein may have inhibitory effects on the survival of cancer cells, particularly leukemic cells such as those that occur in chronic myelogenous leukemia and acute lymphoblastic leukemia.

The ability of compounds to inhibit cancer cell survival can be demonstrated in a variety of ways. Preferred compounds provided herein yield positive results in one or more assays designed to evaluate the cell growth inhibitory properties of compounds. For example, compounds can be evaluated for the ability to inhibit cell growth and/or promote cell differentiaion.

A. Cell Growth Assay

The growth of cancer cells exposed to a compound is evaluated using a fluorometric assay. Cells useful in the assay include human myelogenous leukemic, Philadelphia chromosome-positive cells (K562 cells obtained from American Type Culture Collection, Rockville, MD).

The K562 cells are cultured in RPMI medium containing 10% fetal calf serum, 2 mM glutamin, and 100 μg/ml streptomycin. For the cell growth assay, cells (2-8 X 10^3 cells/well) are incubated with increasing concentrations of compound in a final volume of 200 μl in 96-well plates. Control cells are incubated with medium containing identical concentrations of the solvent dimethylsulfoxide (DMSO) but lacking compound. Cell growth is quantitated after two-to-six days of incubation using a fluorometric assay employing Alamar blue stain [see Page et al. (1993) Int. J. Oncol. 3:473].

Unprocessed fluorescence data from each plate are automatically transferred from a fluorescence plate reader (e.g., Cyto Fluor II, PerSeptive Biosystems) to a computer where the background fluorescence (F) measurements (i.e., complete medium plus stain, but lacking cells) are subtracted from the appropriate control well fluorescence values and where the appropriate test-blank fluorescence measurements (i.e., complete medium plus test compound dilution and stain, but lacking cells) are subtracted from the appropriate test well fluorescence values. The values of the mean ± the standard deviation of the fluorescence measurements from replicate wells are calculated.

Data are expressed in terms of T/C [(F of treated cells/F of control cells) x 100], as a measure of cell viability and survival in the presence of test compound. Using these calculations, it is possible to characterize the effect of the compound, based on the cellular responses, in terms of growth stimulation, growth inhibition or no effect. The concentration of compound that results in 50% inhibition of cell growth (i.e., the IC_{50}) may be determined from the dose-response curve. Thus, the IC_{50} represents the concentration of compound that yields a T/C value of 50%.

In general, treatment of cancer cells, particularly leukemic cells, in the above-described assay with compounds provided herein will result in a decreased growth of the cells as compared to the growth of the same cells that have not been treated with the compounds. Particularly preferred among the compounds provided herein are those that yield comparatively low IC_{50} values in the cell growth assays. The most preferred compounds will yield IC_{50} values that are lower than the IC_{50} value obtained in assays of known reference compounds such as AG490, AG1478. AG1479, AG814, AG946, AG952, AG896, AG953, AG956, and AG957 [see Kaur et al. (1994) Anti-Cancer Drugs 5:213-222]. The reference compound is selected based upon the selected targeted tumor and cell line. For example, when A431 cells, which express high levels of EGF receptors are used, AG1479 is selected as a reference compound. Optionally, the preferred compounds

provided herein have a greater growth inhibitory effect on cancer cells than on non-cancerous cells.

B. Assay of erythroid differentiation

One means by which compounds provided herein may exert an inhibitory influence on cancer cell growth is through induction of erythoid differentiation of leukemic cells. Therefore, the compounds provided herein may also possess differentiation-inducing properties. Such properties may be evaluated as follows.

K562 cells (2-8 x 10^3 cell/well) are incubated with increasing concentrations of compound in a final volume of 200 μl in 96-well plate. Control cells are incubated with medium containing identical concentrations of the solvent, DMSO.

The erythroid differentiation of the cells is assessed by evaluating the level of induction of hemoglobin synthesis after 4 days. At the end of treatment, media are removed by centrifugation of the plates at low speed, and the plates are washed twice with PBS (250 μl/well). Cells are lysed by adding 50 μl PBS containing 1% NP-40 to each well and incubating the plate at room temperature for 30 min. The hemoglobin levels in the cell lysates are measured in the 96 well-plate using a photometric method employing bemzidine [see e.g., Villeval et al.. (1983) Exp. Cell Res. 146:428].

C. Protein Tyrosine Kinase Assay

Universal kinase assays are provided herein. The following assay, while exemplified with respect to $p60^{c\text{-}src}$ and used for screening for inhibitors thereo can be adapted for screening for any selected PTK by appropriate choice of substrate and enzyme.

Materials and Methods

Materials

Strepavidin covalent ScintiStrips were obtained from Wallac, Inc. (Gaithersburg, MD). The random polymer poly [Glu, Tyr]$:1, AStP, dimethylsulfoxde (DMSO), dithithreitol (DTT) and bovine serum albumin (BSA) were obtained from Sigma (St. Louis, MO); tyrphostin controls and

NHS-biotin were obtained from CalBiochem (San Diego, CA); and [γ^{32}P]ATP (specific activty 1000-3000 Ci/mmol) was obtained from Amersham (Arlington Heights, IL). Human recombinant p60$^{c\text{-}src}$ was obtained from Upstate Biotechnology (Lake Placid, NY). All other reagents were obtained from Fisher Scientific.

Biotinylation of poly[Glu, Tyr]4:1

NHS-biotin was used to biotinylate poly[Glu, Tyr]4:1 (5 mg) at the amino terminus folllwoing the manufacturer's instructions. The unreacted biotin-NHS was removed using a BioRad BioSpin 6 column (BioRad, Hercules, CA), and the product eluted into 50 mM Tris-HCl, pH 7.4.

Coating scintillating microplates with random polymer substrate

Strepavidin covalent ScintiStrips were treated with 100 mL/well of a coating solution containing 0.1 mg/mL biotinylated poly[Glu, Tyr]4:1 in 50 mM Tris, pH 7.4, 150 mM NaCl. After an overnight incubation at room temperature, the wells were washed with 3 x 300 μL of assay buffer (50 mM Tris-HCl, pH 7.4, 4.5 mM $MnCl_2$, 0.14 mM Na_3VO_4, 1.4 mM DTT, 0.14 mg/mL BSA) just prior to use.

Preparation of tyrphostin solutions

Stock solutions (5.0 mM) were prepared by weighing tyrphostin controls in glass vials and dissolving in DMSO. A stock solution of hte standard compound tyrphostin AG490, was similarly prepared. These solutions were diluted into 50 mM Tris, pH 7.4, to produce 1000, 500, 100 and 10 μM concentrations.

Tyrosine kinase assay

Assay buffer (70 μL) was added to each well, which was coated with substrate. The p60$^{c\text{-}src}$ enzyme was diluted to 0.175 mg/mL in 50 mM Tris, pH 7.4. Volumes of 10 μL tyrphostin compound solution or 50 mM Tris, pH 7.4, 10 μL 30 μM ATP containing 40 μCi/mL[γ^{32}P]ATP, and 10 μL enzyme solutions were added to the wells. The final concentrations of the tyrphostins were: 100, 50, 10 and 1 μM. After incubating the plate for 5 h,

the wells were washed with 3 x 3000 μL wash buffer (2M NaCl, 10 mM $Na_4P_2O_7$, pH 7.4) followed by 1 x 100 μL 70% EtOH. The wells were air dried and the plates were counted on a Wallac 1450 MicroBeta Trillux counter.

D. Results

Selected compounds from the libraries provided herein were tested in these assays. Results are summarized in FIGURE 42. Exemplary compounds that were synthesized and tested are set forth in FIGURE 41.

EXAMPLE 9

Radiation grafting of a polymer on an inert surface for preparation of matrices with memories

Matrices for use as supports for synthesis and for use in coupled [single platform] protocols have been prepared using radiation grafting. These supports include any inert surface, including PFTE [TEFLON®], which heretofore does not appear to have been used for radiation grafting. The methods exemplified below with reference to FIGURES 34 have been designed for use with PFTE as well as other surfaces. A method of radiation-induced grafted copolymerization of styrene to Teflon (PTFF) has been developed.

A. Scheme 1

1. Preparation of polymer

Scheme 1 shows the preparation of polymer. Polystyrene is radiation grafted onto polypropylene or TEFLON®
tubes, an RF tag, such as the BMDS tag, or IDTAG transponder, was inserted into the tube to produce what will be provided under the name MICROTUBE. The polystyrene is then functionized with selected functional groups [i.e., such as "A" in FIGURE 34A]. Scintillant is covalently linked onto the polystyrene though "A", and a bioactive molecules, such as, for example, biotin, can be synthesized on the surface using the remaining "A" functionalities.

2. **Radiation**

The teflon (PTFE) tube was radiated under a Co^{60} source at a dose rate of $0.1x10^5$ r/h; the total dose is typically $2.6\text{-}2.9x10^6$r.

3. **Polymers**

Using radiation-induced grafting polymerization techniques, a variety of monomers such as styrene, acrylic acid, methylacrylic acid, 2-hydroxy-methylacrylate, and other such monomers can be used to produce different polymeric surfaces with different functional groups on polypropylene (PP), polyethylene (PE) and fluoropolymers. Polyethylene oxide (PEG) may be grafted onto the surface to change the hydrophlicity and reduce the steric-hinderance to antibodies or receptors. Functional groups such as amines, alcohols and phenols, carboxylic acids, halides, aldehydes, nitriles and and other such groups. can be introduced.

It was found that dilution of monomers, such as styrene, with methanol enhanced the rate of grafting PP and PTFE tubes have demonstrated highest styrene grafting at styrene concentrations of about 25 to 50%.

4. **Functionalization**

The functionalization was performed using the readily available N-(hydroxymethyl) phthalimide, with trifluoromethanesulfonic acid as catalyst. The polystyrene grafted tubes is thoroughly washed before use to remove residual monomer, non-attached polystyrene and additives remaining from radiation grafting. The amidoalkylation proceeds smoothly in the 50% (v/v) trifluoroacetic acid - dichloromethane as solvent at room temperature for 24 hours. The predetermined loading can be obtained by changing the concentrations of reagent, catalyst and reaction time. The hydrazinolysis in refluxing ethanol gives the aminomethyl polystyrene grafted PTFE tube.

The MICROTUBE microreactors were prepared in different sizes (2-12mm) with loading capacity range from 0.5 - 15 ymol per tube.

5. **Fluorophores**

The scintillants, which are chemical stable, were chosen to match the energy gap from radiation energy of radioisotopes. Scintillants such as 9-anthracenepropionoc acid, 1-pyrenebutanoic acid and their derivatives are matched to the energy transfer for different radioisotopes, in including ^{125}I, ^{3}H, ^{14}C and others. Care should be taken when selecting combinations of scintillants and radioisotopes to match so that energy transfer from isotope to scintillant is matched.

A portion of the functional groups were covalently linked to the mixture of primary fluor (S1, molecules that emit light following interaction with radiation) and secondary fluor (S2, wavelength shifter). Experiments were performed with mixture of S1/S2 at the ratio ranging from 20:1 to 100:1 for S1 and S2 respectively, with optimum ratio of 40:1 for most of the experiments presented here. Conditions in which 20% to 80% of the functional groups were occupied with mixture of S1/S2 were evaluated. The optimum number of the functional group linked to primary and secondary fluors for most of the experiments was 50%.

The remaining of the functional groups (20% to 80%) were used for chemical synthesis. Small molecules (e.g., biotin) were synthesized on the solid support as described in the scheme 2.

6. **Chemical synthesis on the surface of MICROTUBE:**

A variety of small molecules, such as biotin, peptides, and oligonucleotides, may be synthesized on the MICROTUBE microvessel [see, e.g., scheme 2 (biotin), below]. In order to reduce steric hinderance and improve the interaction of labeled biological target (e.g. antibody, receptor, and complementary DNA or RNA, labeled probe), and depending on the size and nature of the small molecule, different percentages of the functional groups were used for chemical synthesis while the remaining functional group(s) were blocked with Boc. Conditions in which 0.25% to 100% of the functional groups were used for chemical synthesis were evaluated.

The results indicated that use of 25% of the functional groups for chemical synthesis is optimal.

B. Scheme 2: Biotin synthesis

In order to reduce steric hinderance and improve the interaction of labeled biological target [e.g., ^{125}I-receptor), and depending on the size and nature of the small molecule, a different percentage of the functional groups was utilized for chemical synthesis, while the remaining functional group were blocked with Boc. The experiments indicate that optimum results are obtainable with 25% of the functional group dedicated for chemical synthesis.

1. Synthesis

Fmoc (Fmoc-Gly-OH) and Boc(Boc-Gly-OH) linked amino acids were used to control the loading of scintillants and remaining amines. The Fmoc groups were removed using 20 piperidine in DMF, and Boc groups were removed using 1:1 ratio of TFA and dimethylmethane. 50% amine groups were covalently linked to scintillants. The remaining 50% amine were used to synthesis biotin.

2. Assays

The activity of molecules synthesized ont he surface of the microvessels may be evaluated in a variety of solid based assay formats.

a. SPA Assay

The biological activity of small molecules synthesized on the surface of the tubular matrices with memories may be evaluated in a variety of scintillation proximity assay formats as described herein. For example, biotin and its derivative (2-imidazolidone-4-carboxylic acid) were synthesized on the tube and the binding characteristics of the synthesized molecules on the solid support to 125I-streptavidin in scintillation proximity assay were evaluated. The results demonstrated that biotin derivative (2-imidazolidone-4-carboxylic acid) that has much lower affinity for streptavidin exhibited a lower signal.

b. **ELISA type assay**

ELISAs can be performed using antibodies to small molecules, such as a peptide. For example metenkephalin was synthized on the MICROTUBE microvessed, and anti-metenkephalin antibody was used. As an example of nonpeptide small molecule biotin was syntheized and an anti-biotin antibody labeled with alkaline phosphatase was used to detect by colorimetric, fluorometric or luminescent means.

c. **Radio-immunoassay**

Using radio-labeled antibody or receptor, a variety of radio-immunoassays may be designed usig the microvessels, such as the MICROTUBE microreactors.

C. **Radiation grafting**

A a teflon tube [19 mm, long, OD:5mm, ID:2mm; see FIGURES 34C and 34D] is radiation grafted. It was found that dilution of styrene with methanol enhances the rate of grafting. Dilutions of from 5% to 70 % were tested. The PTFE tube has the highest styrene grafting at a 50% dilution [in contrast, a polypropylene tube has the best performance at 35% dilution. The teflon (PTFE) tube is radiated under Co^{60} source at a dose rate of 0.1 x 10^6 r/h; the total dose of 2.6-2.9 x 10^6 r.

Functionization is performed using N-(hydroxymethyl) phthalimide, with trifluoromethanesulfonic acid [TFMSA] as a catalyst. The polystyrene grafted PTFE tube is thoroughly washed before use to remove residual monomer, non-attached polystyrene and additives remaining from radiation grafting. The amidoalkylation proceeds smoothly in the 50 % (v/v) trifluoroacetic acid - dichloromethane solvent at room temperature for 24 hours. The predetermined loading can be obtained by changing the concentrations of reagent, catalyst and reaction time. The hydrazinolysis in refluxing ethanol gives the aminomethyl polystyrene grafted PTFE tube.

FIGURE 34 depicts the protocol for radiation grafting of polymers to the surface of TEFLON® [or other suitable surface]. FIGURE 34C depicts the preparation of a tubular devices in which the matrix is the radiation grafted

PTFE and the memory is a transponder, such as the monolithic device provided herein, the BMDS transponder or IDTAG™ transponder [such as a MICROTUBE], described herein; and FIGURE 34D depicts the small chip [about 2-3 mm x 2-3 mm x 0.1-0.3 mm, see, FIGURES 43 and 44] encased in a radiation grafted polyprolene or teflon ball [or bead, conical tube or other such geometry] with a screw cap or snap on lid [such as device provided herein that will be provided under the name MICROBALL or MICROBEAD or MICROTUBE]. These devices may have removable lids, such as a snap on lid, preferably a snap on lid, or a screw top, so that the memory device can be removed and reused, and can be added after radiation grafting. Loading on the grafted tubes and balls is adjustable can was typically about 0.5 - 15 μmol per tube. The amount can be varied by altering the size of the tube or balls. A variety of selected functional groups are available. Any known to those of skill in the art may be used, including any described herein. PFTE devices are particularly suitable for high temperature reactions [loading was less than or about 3 μmol per device].

D. Protocol for Increasing Loading on Fluoropolymer

In the radiation-induced grafted copolymerization of styrene to ETFE and Teflon (PTFE) tube (21 mm long, OD:6mm, ID: 4mm), the dilution of styrene with methanol enhances the rate of grafting. Dilutions of from 5% to 70% and the PTFE tube has the highest styrene grafting at a 50% dilution. By adding a mineral acid such as sulfuric acid and nitric acid (concentrations from 0.01 - 0.5 M), the polystyrene grafting was increased from 50 - 200%. See table below.

The functionization was performed as described above, using N-(hydroxymethyl) phthalimide, with trifluoromethanesulfonic acid as catalyst. The polystyrene grafted PTFE tube was thoroughly washed before use to remove residual monomer, non-attached polystyrene and additives remaining from radiation grafting. The amidoalkylation proceeded smoothly in the 50% (v/v) trifluoracetic acid - dichloromethane as the solvent at room temperature for 24 hours.

A predetermined loading could be obtained by changing the concentrations of reagent catalyst and reaction time. The hydrazinolysis in refluxing ethanol gave the aminomethyl polystyrene grafted PTFE or ETFE tube. The loading of PTFE was about 20 micromol, and ETFE was as high as 45 micromol.

Acid Concentration (M)	Polystyrene attached on tube surface (mg) PTFE	Polystyrene which attached on tube surface (mg)
0	10	19
0.05	12	32
0.1	12	33
0.2	20	35

EXAMPLE 10

Wash and SPA assay procedure using MICROTUBE microreactors

1. Covalently linking scintillant to the surface of the MICROTUBE microreactor

Scintillants (pyrenebutyric acic and 9-anthracenepropionic acid) were covalently linked to the grafted polystyrene on the surface of the polymer. The Fluorophore was linked to 50% of the available functional groups as described above (see polymer preparation).

2. Synthesizing biotin on the MICROTUBE microreactor

The remaining 50% functional amine groups on the surface of the MICROTUBE microreactor was estimated by Fmoc to be ~1.8 μmol/tube. The amine group was covalently linked to biotin under conditions described below. 0.012 M biotin, 0.024 MDiEA (diisopropylethylamine), 0.012 M PYBOP (Benzotriazol-1-yl-oxy-tris-pyrrollidino-phophonium hexafluorophosphate) in DMF (N,N-Dimethyl foramide) at room temperature for 1 hour.

3. Washing protocol for MICROTUBE microreactors

A. Development and Optimization of wash procedure.

The MICROTUBE microreactors were washed with various detergents (SDS, CHAPS, Triton X-100, or Benzalchonium Chloride) or charcoal. The effects of detergents were evaluated by washing the microreactors with different concentrations of detergents (0.5 to 5% in PBS) for 24 hours on an orbital shaker at room temperature. The charcoal wash was done by dialysis against PBS containing 10-35% charcoal (4-8 mesh).

It was found that the MICROTUBE microreactors that had been washed with SDS, Benzalchonium Chloride or charcoal had an improved signal. Additional wash studies were performed with either SDS and/or charcoal in wash buffer. The effect of SDS concentration was assessed by washing the tube with 0.25, 0.5, 0.75, or 1% SDS in PBS for 24 hours. Results of this experiment indicated that microreactors that had been washed with 0.5%-0.75% SDS and/or charcoal in PBS yielded a better signal.

Finally, the optimal wash period was determined by washing microreactors with 0.75% SDS/charcoal for 1, 2, 3, 4, or 5 days at room temperature on an orbital shaker. The results of this experiment revealed that washing tubes for 2 days efficiently removes undesirable material which interfere with the SPA signal.

B. Optimized Wash Procedure.

After synthesis of small molecules (biotin) on the MICROTUBE microreactors were washed as described above. The MICROTUBE microreactors were placed in a dialysis bag and were dialyzed against PBS containing 0.75% SDS +/- 35% charcoal for 2 days at room temperature on an orbital shaker. At the end of SDS wash, microreactors were rinsed with PBS (10 ml/MICROTUBE) 2 times.

Thus, performance of assays on solid supports can be improved by washing the solid support with linked biological particle or molecule with 0.75% SDS with or without 35% charcoal in PBS (pH 7.2) for about 2 days.

2. **Blocking**

The MICROTUBE microreactors were placed in PBS (pH 7.2) buffer containing 3% BSA (bovine serum albumin) and incubated overnight at 4° C

3. **SPA Detection.**

Biotin was detected in the SPA format. MICROTUBE microreactors were placed in 24 well plate containing 1 ml of Assay Buffer [10 mM Sodium Phosphate pH 7.2, 150 mM NaCl, 0.5% BSA, 0.05% Tween 20, and ^{125}I-streptavidin (244 ng/ml, specific activity 0.291 μCi/μg)]. MICROTUBE microreactors were incubated at room temperature on an orbital shaker for 2 hours. The extent of ^{125}I streptavidin binding on the MICROTUBE microreactors was assessed in a Wallac MicroBeta Trilux scintillation counter.

EXAMPLE 11

Synthesis of libraries on OMDs

Libraries are synthesis on OMDS [described above, see, e.g., FIGS. 22-30 and 33]. Although this example is provided with reference to oligonucleotides, it is understood that the general strategy is applicable to synthesis of the compounds provided herein. Instead of synthesizing oligonucleotides, the compounds and libraries herein are synthesized on the OMDs.

Referring to FIG. 33, polypropylene sheets [(10x10x1 mm) the Moplen resin e.g., V29G PP resin from Montell, Newark DE, a distributor for Himont, Italy] are radiation grafted with polystyrene to give the surface modified devices1 [MACROCUBES™ or MACROBEADS™]. Each such device is imprinted with a unique symbology, such as the two-dimensional optical bar code using the methods described herein. The OMDs [also called laser optical synthesis chips] are then subjected [see, FIG 33] to a modified aminomethylation procedure [Mitchell et al. (1978) J. Org. Chem. 43:2845; Mitchell et al. (1976) J. Org. Chem. Soc. 98:7357; or other procedures to obtain other functional groups(Farrall et al. (1976) J. Org. Chem. 41:3877; Merrifield et al. (1985) Angew. Chem. Int. Ed. Engl. 24:799])] to

functionalize the polystyrene surface graft. Procedures are exemplified in the examples.

A laser optical memory device is shown in Figure 33A and 33B. It was fabricated by combining two components: a 2-dimensional (2-D) 16 digit bar code for encoding and a separate polymeric support for synthesis. The 2-D bar codes were laser-etched by a CO_2 laser on 6 X 6 segments of a chemically inert alumina ceramic plate (Coors Cermaics, thickness = 0.5 mm; the actual size of each 2-D bar code is 3 X 3 mm). The surrounding synthesis support is a stable polypropylene or fluoropolymer square (10 X 10 X 2 mm) radiolytically grafted [as describe herein] with low cross-linking polystyrene [Battaerd, G. W. Tregear, (1967) in Graft Copolymers, John Wiley & Sons, Interscience, New York] and designed with a square hole (6 X 6 mm) in the middle. The etched ceramic block is securely inserted within the hole to form the entire OMD. Very small size OMDS can be manufactured as the laser etching optical resolution of an entire 2-D bar code can extend well below 0,5 mm in total diameter. 2-D bar coding has the advantage over regular linear bar coding of more data compression in a much smaller surface area (Fig. 33B).

A loading of a 5-8 μmol/device was typically obtained as measured by Fmoc analysis. At this point, the OMDS are ready for use in combinatorial or standard chemical synthesis.

A directed sorting strategy [instead of statistical pool and splitting] was used in the construction of combinatorial libraries with zero redundancy [i.e., the number of OMDs is equal to the number of the library members]. In an example of a 3 X 3 directed sorting synthesis [FIG 33C), nine OMDs are first scanned optically using a small camera [i.e., such as the QuickCam™]linked to the pattern recognition software [see, description above and FIG. 31] on a computer, and each device [with a unique 2-D bar-code], i.e., 1-9, for exemplifiation, is assigned to one of the nine members in the library [a Code-Structure Table] by the software that directs the sythesis, such as the Synthesis Manager software [see, description above

and in the EXAMPLES]. The OMDs are then split [sorted], using software for synthesis and for decoding the 2-D code pattern [see description herein] into three groups according to the first building block (A, B, or C) for each structure as pre-assigned in the Code-Structure Table. A reaction with building block A, B, or C is then performed on each specific group. The OMDs are then pooled, washed, subjected to common reactions, scanned and re-sorted into three new groups according to the second building block (A, B, or C). A second reaction with building block A, B, or C is then performed with each group of OMDs. The OMDs can then be pooled again, subjected to common manipulations, and sorted. The process is repeated until the synthesis is completed.

The structure of the compound synthesized on each OMD can then be de-coded simply by optically reading the 2-D image with synthesis software via the camera and the decoding software and correlating the bar code with the structures in the Code-Structure Table.

To demonstrate the use of the laser optical synthesis OMDs in large scale synthesis, a library of 27 oligonucleotides with a general structure of X_4-X_3-X_2-T was constructed using the above described Directed Sorting strategy (FIGURE 33D). Since polystyrene does not completely swell in acetonitrile or water, which are the solvents for the coupling and cleavage steps in a standard oligonucleotide synthesis cycle[Gait et al. (1990) in Oligonucleotide Synthesis, A Practical Approach, Gait, Ed., IRL Press, Oxford], reaction conditions were modified to accommodate the polystyrene support. Among the range of solvents and co-solvents investigated, it was found that a mixture of acetonitrile and dichloromethane (2:3, v/v) for the coupling reaction gave the highest coupling efficiency, and water / 1,4-dixane (1:1, v/v) performed best as the solvents for the cleavage step. The standard conditions for the de-blocking step (3% trichloroacetic acid / dichloromethane) and the oxidation step (0.1 M I_2/THF) are directly applicable. All reactions [see FIG 33D] were performed in appropriate size

glass bottles with Teflon-linered screw caps, and all the washings are performed using acetonitrile and dichloromethane alternately.

Reaction conditions were as follows:

de-blocking, 3% TCA/DCM, rt, 2 min, erpeat 2 times, coupling, 0.1 M DMT-X_n, 0.3 M tetrazole, ACN/DCM (2:3 v/v), rt, 1 h; oxidation, 0.1 M I_2, THF/pyridine/H_2O (40:10:1, v/v/v), rt, 20 min; capping, 0.5 M Ac_2O, 0.5 M 1-methylimidazole, 0.45 M 2, 6-lutidine, THF, rt, 20 min.; cleavage, concentrated ammonia/1,4-dioxane (1:1 v/v), rt, 20 h; deprotection, concentrated ammonia, 55 ° C, 20 h,; de-salting (and de-blocking) on Poly Pak cartridges (according to manufacturer's instructions).

Twenty-seven amino-functionalized OMDs were first reacted with 0.1 M 5'-O-DMT-3'-succinic acid-2'-deoxythymidine / 0.1 M PyBop/ 0.2 M DIEA / DMF (30 ml) at room temperature for 4 hours. The OMDs were washed (acetonitrile and dichloromethane alternately, 30 ml x 4 for each solvent) and dried under vacuum at room temperature for 30 min (all subsequent reactions were followed by the same washing and drying procedures). The OMDs were then capped with Ac_2O/ 1-methylimidazole / 2,6-lutidine / THF (0.5 M for each, 30 ml) at room temperature for 20 min., and de-blocked with 3% trichloracetic acid / dichloromethane (30 ml, rt, 2 min., repeated two times). UV measurement of the de-blocking solution indicated an average loading of 7.0 μmol per OMD. The OMDs were then scanned and each OMD was assigned to one of the 27 oligonucleotide sequences in the library (Code-Structure Table) using Synthesis Manager and QuickCam. The OMDs were sorted into three groups according to the second residue assignment (**A, G,** *or* **C**) and coupled with one of the corresponding β-cyanoethyl phosphoramidites (0.1 M) in acetonitrile / cichloromethane (2:3, v/v, 10 ml, with 0.3 M tetrazole) at room temperature for 60 min. The OMDs were then pooled together, oxidized (0.1 MI_2 / THF / pyridine / H_2O, 30 ml, rt, 20 min), capped, and de-blocked. Next, the OMDs were subjected to another cycle of sorting-->coupling-->oxidation-->capping-->de-blocking according to the third residue (X_3) in their assigned structures.

After the third residue, the OMDs were scanned, sorted into three groups according to their last residues (X_4), coupled to the corresponding phosphoramidites, and oxidized. The capping and de-blocking steps were omitted in this cycle.

The washed OMDs were then scanned with the QUICKCAM® and the sequence of the oligonucleotides on each OMD was de-coded. Each OMD was put into a 5 ml glass vial labeled with the corresponding oligonucleotide sequence. Concentrated ammonia and 1,4-dioxane (2 ml, 1:1, v/v) was added to each vial and the vials were sealed with Teflon-linered screw caps. The vials were shaken at room temperature for 20 hours to cleave the oligonucleotides from the support. The OMDs were then removed from the vials and rinsed with aqueous dioxane (0.5 ml x 2) and the vials were evaporated to dryness under vacuum. Next, fresh concentrated ammonia (2 ml each) was added to each vial. The vials were capped tightly and heated in an oil bath (55°C) for 20 hours to removed the cyanoethyl, isobutyryl, and benzoyl groups. An aliquot (10 μl) of the crude oligonucleotide solution from each vial was saved for HPLC analysis and the rest of the solutions were evaporated under vacuum. The crude oligonucleotides were then de-blocked and de-salted with Poly Pak™ cartridges (Glenn Research) using standardized procedures[User Guide for Poly Pak Cartridges from Glenn Research]. The fully de-protected oligonucotides were lyophilized from 20% acetonitrile / water (white solids), weighed, and analyzed by MS, ^{1}H NMR:

[Selective ^{1}H NMR spectra data (500 MHz, D_2O). **ACCT** (entry 25, Table 1): s=8.47 (s, 1 H, CH-adenine), 8.37 (s, 1 H CH-adenine), 8.09 (d, J= 7.8 Hz, 1 H, CH-cytosine), 8.06 (d, J=7.8 Hz, 1 H, CH-cytosine), 7.68 (s, 1 H, CH-thymine), 6.15-6.32 (3 multiplets, 6 H, CH-cytosine and O-C(N)H), 3.81-5.03 (6 multiplets, 16 H, O-CH_2 and O-CH), 2.28-2.92 (4 multiplets, 8 H, CH_2), 1.87 (s, 3 H, CH_3). **ACAT** (entry 7, Table 1): s=8.47 (s, 1 H, CH-adenine), 8.44 (s, 1 H CH-adenine), 8.35 (s, 1 H, CH-adenine), 8.34 (s, 1 H, CH-adenine),8.05 (d, J = 8.0 Hz, 1 H, CH-cytosine), 7.59 (s, 1 H, CH-thymine), 6.15-

6.43 (3multiplets, 5 H CH-cytosine and O-C(N)H), 3.78-5.1 (7 multiplets, 15 H, O-CH_2 and O-CH), 2.17-2.87 (5 multiplets, 8 H CH_2), 1.79 (s, 3 H, CH_3).]

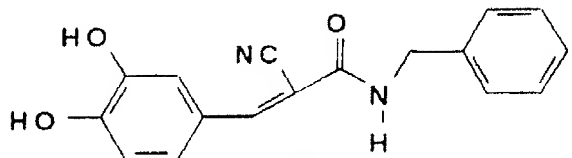
The OMDs were also analyzed by sequence analysis. Oligonucleotide sequence analysis was performed using electrospray and EM mass spectrometry [ES MS-MS; see, e.g., Siuzdak (1966) in Mass Spectrometry for Biotechnology, Academic Press, San Diego; Metzger et al. (1994) Anal. Biochem. 219:261; Ni et al. (1996) Anal. Chem. 68:1989].

Each oligonucleotide gave the expected molecular ion in mass spectroscopy analysis as configured by the 2-D encoding. Sequence analysis of two oligonucleotides with the same molecular weight showed that they had the expected sequences. The crude products had good to excellent purity as analyzed by reverse phase HPLC. A quick, standard de-salting procedure using Ply Pak cartridges yielded 27 pure (>95% by HPLC) oligonucleotides with good overall isolated yields (while solid, 2-7 mg each). These data show that the combinatorial strategy of oligonucleotide synthesis using the laser optical synthesis (LOSC) technology should produce a large number and quantity of oligonucleotides with high purity.

Since modifications will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

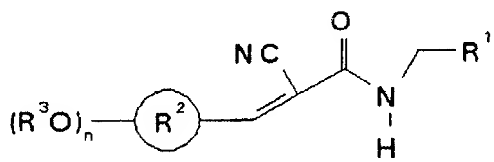
CLAIMS:

1. A composition of compounds, comprising analogs of tyrphostin AG490, wherein tryphostin AG490 has the structure:



AG 490

2. A compound that has the formula(I):



,

wherein:

n is 0 to 3;

R^1 is selected from the group consisting of alkyl, which may be straight or branched or cyclized containing one ring or two fused rings, or mixtures thereof, containing preferably 1 to 15 carbons in the chain, aryl or heteroaryl, containing one ring or two fused rings and 5 to 7 members in the ring; R^1 is unsubstituted or is substituted with one or more substituents selected from alkyl, cyano, halide, alkoxy, alkoxyalkyl, alkyl, thioxy, alkylthioxy, haloalky, OH, and amino;

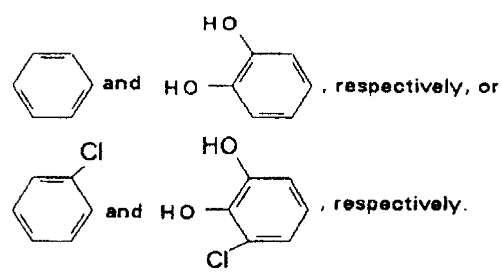
R^2 is selected from the group consisting of alkyl, which may be straight or branched or cyclized containing one ring or two fused rings, or mixtures thereof, containing 1 to 15 carbons in the chain, and aryl or heteroaryl, containing one ring or two fused rings and 5 to 7 members in the ring; R^2 is unsubstituted or is substituted with one or more substituents selected from $(R^4)_p$ where p is 0 to 3,

R^3 is selected from H, $(CH_3)(CH_2)_qC(O)$, aryl-$(CH_2)_qC(O)$ or heteroaryl-$(CH_2)_qC(O)$, wherein the aryl or heteroaryl groups are unsubstituted or are

substituted with the any of the groups set forth for R^4 and contain single rings or two fused rings containing 5 to 7 members in a ring;

each R^4 is independently selected from alkyl, cyano, halide, alkoxy, alkoxyalkyl, alkyl, thioxy, alkylthioxy, haloalky, OH, amino, phenoxy, alkylenedioxy, alkylenethioxyoxy and alkylenedithioxy; and

the compounds are selected with the proviso that if R^3 is H, then R^1 and R^2 are not:



3. The compounds of claim 2, wherein the alkyl groups contain 1 to 6 carbons.

4. The compounds of claim 2, wherein:

R^1 and R^2 are each independently aryl or heteroaryl containing 5 to 7 members in the ring; and

R^4 is OH or halide or lower alkyl or alkoxy, and n is preferably 0 to 2

5. The compounds of claim 4, wherein R^3 is acetyl, benzoyl or H.

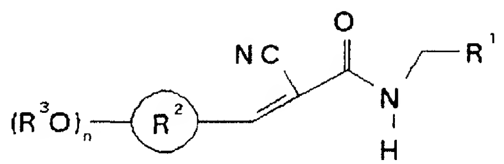
6. The compounds of claim 2, wherein R^1 and R^2 are phenyl groups.

7. The compounds of claim 2, wherein each of R^1 and R^2 are independently selected from the group consisting of phenyl, thienyl, furyl, pyrrolyl, phenyl, pyridinyl, morpholino, or pyrimidinyl.

8. A compound of claim 2 selected from the group consisting of the compounds set forth in Figure 40.

9. A composition, comprising at least two compounds of claim 2.

10. A composition, comprising at least two compounds having formula (I):



,

wherein:

n is 0 to 3;

R^1 is selected from the group consisting of alkyl, which may be straight or branched or cyclized containing one ring or two fused rings, or mixtures thereof, containing preferably 1 to 15 carbons in the chain, aryl or heteroaryl, containing one ring or two fused rings and 5 to 7 members in the ring; R^1 is unsubstituted or is substituted with one or more substituents selected from alkyl, cyano, halide, alkoxy, alkoxyalkyl, alkyl, thioxy, alkylthioxy, haloalky, OH, and amino;

R^2 is selected from the group consisting of alkyl, which may be straight or branched or cyclized containing one ring or two fused rings, or mixtures thereof, containing 1 to 15 carbons in the chain, and aryl or heteroaryl, containing one ring or two fused rings and 5 to 7 members in the ring; R^2 is unsubstituted or is substituted with one or more substituents selected from $(R^4)_p$ where p is 0 to 3,

R^3 is selected from H, $(CH_3)(CH_2)_qC(O)$, aryl-$(CH_2)_qC(O)$ or heteroaryl-$(CH_2)_qC(O)$, wherein the aryl or heteroaryl groups are unsubstituted or are substituted with the any of the groups set forth for R^4 and contain single rings or two fused rings containing 5 to 7 members in a ring; and

each R^4 is independently selected from alkyl, cyano, halide, alkoxy, alkoxyalkyl, alkyl, thioxy, alkylthioxy, haloalky, OH, amino, phenoxy, alkylenedioxy, alkylenethioxyoxy and alkylenedithioxy.

11. The composition of claim 1, wherein each compound is linked to a solid support.

12. The composition of claim 11, wherein the solid support is combined with a memory.

13. The composition of claim 12, wherein the memory is encased in the support.

14. The composition of claim 12, wherein the memory is an electromagnetic tag.

15. The composition of claim 12, wherein the memory is an optical memory.

16. The composition of claim 10, wherein each compound is linked to a solid support.

17. The composition of claim 16, wherein the solid support is combined with a memory.

18. The composition of claim 17, wherein the memory is encased in the support.

19. The composition of claim 17, wherein the memory is an electromagnetic tag.

20. The composition of claim 17, wherein the memory is an optical memory.

21. A process for the synthesis of a compound in the composition of claim 1, comprising:

linking an aldehyde R^1CHO to a solid support by way of a reductive alkylation;

cyanoacetylating the resulting product followed by an aldol condensation with $OHC\text{-}R^2\text{-}(OH)_n$; and

the, optionally, esterifying the resulting product with R^3X,

wherein

R^1-CHO is any aldehyde;

$OHC\text{-}R^2\text{-}(OH)_n$ is any aldehyde;

R^3-X is an alkylating or acylating reagent.

22. The process of claim 21, wherein

R^1-CHO is an aromatic or heterocyclic aldehyde;

$OHC\text{-}R^2\text{-}(OH)_n$ is an aromatic or heterocyclic aldehyde with one or more hydroxy groups; and

R^3X is an alkylating or acylating reagent comprising aromatic or heterocyclic rings.

23. The process of claim 21, wherein linkage to the solid support is via an acid cleavable linker.

24. The process of claim 21, wherein the solid support is combined with a memory.

25. The process of claim 24, wherein the memory is encased in the support.

26. The process of claim 24, wherein the memory is an electromagnetic tag.

27. The process of claim 24, wherein the memory is an optical memory.

28. The process of claim 21, wherein:

R^1 is selected from the group consisting of alkyl, which may be straight or branched or cyclized containing one ring or two fused rings, or mixtures thereof, containing preferably 1 to 15 carbons in the chain, aryl or heteroaryl, containing one ring or two fused rings and 5 to 7 members in the ring; R^1 is unsubstituted or is substituted with one or more substituents selected from alkyl, cyano, halide, alkoxy, alkoxyalkyl, alkyl, thioxy, alkylthioxy, haloalky, OH, and amino;

R^2 is selected from the group consisting of alkyl, which may be straight or branched or cyclized containing one ring or two fused rings, or mixtures thereof, containing 1 to 15 carbons in the chain, and aryl or heteroaryl, containing one ring or two fused rings and 5 to 7 members in the ring; R^2 is unsubstituted or is substituted with one or more substituents selected from $(R^4)_p$ where p is 0 to 3,

R^3 is selected from H, $(CH_3)(CH_2)_qC(O)$, aryl-$(CH_2)_qC(O)$ or heteroaryl-$(CH_2)_qC(O)$, wherein the aryl or heteroaryl groups are unsubstituted or are substituted with the any of the groups set forth for R^4 and contain single rings or two fused rings containing 5 to 7 members in a ring;

each R^4 is independently selected from alkyl, cyano, halide, alkoxy, alkoxyalkyl, alkyl, thioxy, alkylthioxy, haloalky, OH, amino, phenoxy, alkylenedioxy, alkylenethioxyoxy and alkylenedithioxy.

29. The process of claim 21, wherein:

each of R^1-CHO, OHC-R^2-$(OH)_n$, and R^3-X are selected from the groups set forth for each in any of FIGURES 37-39.

30. The process of claim 29, wherein the groups are those set forth in FIGURE 37.

31. The process of claim 29, wherein the groups are those set forth in FIGURE 38.

32. The process of claim 29, wherein the groups are those set forth in FIGURE 39.

33. The process of claim 21, comprising the reaction scheme set forth in FIGURE 35.

34. A process for the synthesis of composition of claim 1, comprising:

(a) linking an aldehyde R^1CHO to a solid support by way of a reductive alkylation;

(b) cyanoacetylating the resulting product followed by an aldol condensation with OHC-R^2-$(OH)_n$; optionally,

(c) esterifying the resulting product with R^3X;

(d) repeating steps (a)-(c) a plurality of times;

wherein:

R^1-CHO is any aldehyde;

OHC-R^2-$(OH)_n$ is any aldehyde;

R^3-X is an alkylating or acylating reagent.

35. The process of claim 34, wherein:

R^1-CHO is an aromatic or heterocyclic aldehyde;

OHC-R^2-$(OH)_n$ is an aromatic or heterocyclic aldehyde with one or more hydroxy groups; and

R^3X is an alkylating or acylating reagent comprising aromatic or heterocyclic rings.

36. The process of claim 35, wherein the composition comprises N compounds, the process comprising the steps of:

(a) providing N memory with matrix combinations that have been treated to render them suitable for linking molecules;

(b) selecting sets of building blocks designated BB1, BB2, and BB3, wherein;

each member of BB1 has the formula R^1-CHO and the number of members of BB1 is W;

each member of BB2 has the formula OHC-R^2-$(OH)_n$ and the number of members of BB2 is X;

each member of BB3 is has the formula R^3-X, and the number of members of BB3 is Z; and

$W \times X \times Z = N$;

(b) dividing the memory with matrix combinations into W groups of N/W members and reacting each with one of the W BB1 members, whereby R^1 is linked to the memory with matrix;

(c) before during or after step (b) encoding the memory with information that identifies the BB1 group added to each memory with matrix combination;

(d) pooling the resulting memory with matrix combinations and cyanomethylating the resulting product;

(e) splitting the memory with matrix combination into X groups of N/X members and reacting each with one of the X BB2 members via and aldol condensation;

(f) before during or after step (e) encoding the memory with information that identifies the BB2 group added to each memory with matrix combination;

(f) pooling the resulting memory w matrix combinations, splitting them into Z groups of N/Z members and, except in instances in which BB3 is nothing, reacting each with one of the Z BB3 members;

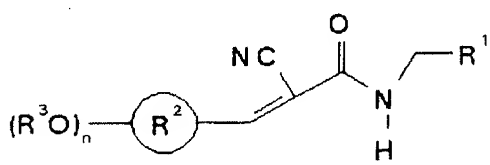
(g) before during or after step (f) encoding the memory with information that identifies the BB3 group added to each memory with matrix combination.

37. The process of claim 36, wherein the linker is an acid cleavable linker and BB1 is linked to an amino group on the linker via an acylation.

38. The process of claim 36, further comprising cleaving the resulting compounds from the support.

39. The process of claim 36, further comprising identifying compounds that exhibit activity as inhibitors of a protein tyrosine kinase.

40. The process of claim 36, wherein the resulting compounds that are linked to the memories with matrices have formula (I):



,

wherein:

n is 0 to 3;

R^1 is selected from the group consisting of alkyl, which may be straight or branched or cyclized containing one ring or two fused rings, or mixtures thereof, containing preferably 1 to 15 carbons in the chain, aryl or heteroaryl, containing one ring or two fused rings and 5 to 7 members in the ring; R^1 is unsubstituted or is substituted with one or more substituents selected from alkyl, cyano, halide, alkoxy, alkoxyalkyl, alkyl, thioxy, alkylthioxy, haloalky, OH, and amino;

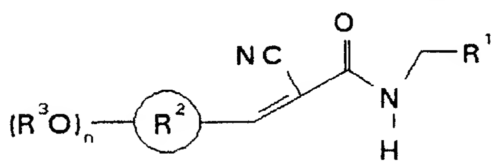
R^2 is selected from the group consisting of alkyl, which may be straight or branched or cyclized containing one ring or two fused rings, or mixtures thereof, containing 1 to 15 carbons in the chain, and aryl or

heteroaryl, containing one ring or two fused rings and 5 to 7 members in the ring; R^2 is unsubstituted or is substituted with one or more substituents selected from $(R^4)_p$ where p is 0 to 3,

R^3 is selected from H, $(CH_3)(CH_2)_qC(O)$, aryl-$(CH_2)_qC(O)$ or heteroaryl-$(CH_2)_qC(O)$, wherein the aryl or heteroaryl groups are unsubstituted or are substituted with the any of the groups set forth for R^4 and contain single rings or two fused rings containing 5 to 7 members in a ring; and

each R^4 is independently selected from alkyl, cyano, halide, alkoxy, alkoxyalkyl, alkyl, thioxy, alkylthioxy, haloalky, OH, amino, phenoxy, alkylenedioxy, alkylenethioxyoxy and alkylenedithioxy.

41. The process of claim 39, wherein the identified compounds are selected from the group of compounds that have formula (I):



wherein:

n is 0 to 3;

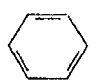
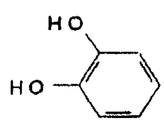
R^1 is selected from the group consisting of alkyl, which may be straight or branched or cyclized containing one ring or two fused rings, or mixtures thereof, containing preferably 1 to 15 carbons in the chain, aryl or heteroaryl, containing one ring or two fused rings and 5 to 7 members in the ring; R^1 is unsubstituted or is substituted with one or more substituents selected from alkyl, cyano, halide, alkoxy, alkoxyalkyl, alkyl, thioxy, alkylthioxy, haloalky, OH, and amino;

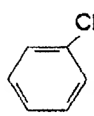
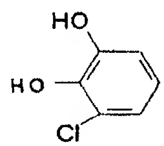
R^2 is selected from the group consisting of alkyl, which may be straight or branched or cyclized containing one ring or two fused rings, or mixtures thereof, containing 1 to 15 carbons in the chain, and aryl or heteroaryl, containing one ring or two fused rings and 5 to 7 members in the ring; R^2 is unsubstituted or is substituted with one or more substituents selected from $(R^4)_p$ where p is 0 to 3,

R^3 is selected from H, $(CH_3)(CH_2)_qC(O)$, aryl-$(CH_2)_qC(O)$ or heteroaryl-$(CH_2)_qC(O)$, wherein the aryl or heteroaryl groups are unsubstituted or are substituted with the any of the groups set forth for R^4 and contain single rings or two fused rings containing 5 to 7 members in a ring;

each R^4 is independently selected from alkyl, cyano, halide, alkoxy, alkoxyalkyl, alkyl, thioxy, alkylthioxy, haloalky, OH, amino, phenoxy, alkylenedioxy, alkylenethioxyoxy and alkylenedithioxy; and

the compounds are selected with the proviso that if R^3 is H, then R^1 and R^2 are not:

 and , respectively, or

 and , respectively.

42. The componds of claim 40, wherein the alkyl groups contain 1 to 6 carbons.

43. The compounds of claim 2, wherein:

R^1 and R^2 are each independently aryl or heteroaryl containing 5 to 7 members in the ring; and

R^4 is OH or halide or lower alkyl or alkoxy, and n is preferably 0 to 2

44. The compounds of claim 40, wherein R^3 is acetyl, benzoyl or H.

45. The compounds of claim 40, wherein R^1 and R^2 are phenyl groups.

46. The compounds of claim 40, wherein each of R^1 and R^2 are independently selected from the group consisting of phenyl, thienyl, furyl, pyrrolyl, phenyl, pyridinyl, morpholino, or pyrimidinyl.

47. The process of claim 36, wherein BB1, BB2 and BB3 are selected from the groups set forth for each in any of FIGURES 37-39.

48. The process of claim 37, wherein N is greater or equal to about 100.

49. The process of claim 37, wherein N is greater or equal to about 400.

50. The process of claim 37, wherein N is greater or equal to about 10,000.

51. The process of claim 37, wherein N is greater or equal to about 100,000.

52. A method for screening for compounds that modulate the activity of protein tyrosine kinases (PTKs), comprising:

adding biotinylated PTK substrate to a microplate containing embedded scintillant and coated with streptavidin;

adding radiolabeled ATP, a test compound and PTK, under conditions whereby labeled phosphate is transferred from the ATP to the bound substrate; and

identifying test compounds that change PTK activity.

53. The method of claim 52, wherein the change in PTK activity is measured by comparing PTK activity in the absence of the test compound or by comparing PTK activity in the presence of the test to the activity in the presence of a compound of known activity.

54. The method or claim 52, wherein the PTK is a Src family member, a Csk family member, a Syk family member, a Btk family member, an Abl family member, an Fps family member, or a Jak family member.

55. The method of claim 54, wherein the PTK is $p60^{src}$.

56. The method of claim 52, wherein the modulation is an inhibition.

57. The method of claim 56, wherein the test compound is a compound of claim 2.

58. The process of claim 39, wherein compounds that exhibit activity as inhibitors of a protein tyrosine kinase are identified by:

adding biotinylated PTK substrate to a microplate containing embedded scintillant and coated with streptavidin;

adding radiolabeled ATP, a test compound and PTK, under conditions whereby labeled phosphate is transferred from the ATP to the bound substrate; and

identifying test compounds that change PTK activity.

59. The process of claim 36, wherein the memory is an electromagnetic tag.

60. The process of claim 36, wherein the memory is an optical memory.

61. The process of claim 36, wherein the memory is encased in the matrix.

62. A library comprising N compounds prepared by a process comprising the steps of:

(a) providing N memory with matrix combinations that have been treated to render them suitable for linking molecules;

(b) selecting sets of building blocks designated BB1, BB2, and BB3, wherein;

each member of BB1 has the formula R^1-CHO and the number of members of BB1 is W;

each member of BB2 has the formula OHC-R^2-$(OH)_n$ and the number of members of BB2 is X;

each member of BB3 is has the formula R^3-X, and the number of members of BB3 is Z; and

$W \times X \times Z = N$;

(b) dividing the memory with matrix combinations into W groups of N/W members and reacting each with one of the W BB1 members, whereby R^1 is linked to the memory with matrix;

(c) before during or after step (b) encoding the memory with information that identifies the BB1 group added to each memory with matrix combination;

(d) pooling the resulting memory with matrix combinations and cyanomethylating the resulting product;

(e) splitting the memory with matrix combination into X groups of N/X members and reacting each with one of the X BB2 members via and aldol condensation;

(f) before during or after step (e) encoding the memory with information that identifies the BB2 group added to each memory with matrix combination;

(f) pooling the resulting memory w matrix combinations, splitting them into Z groups of N/Z members and, except in instances in which BB3 is nothing, reacting each with one of the Z BB3 members;

(g) before during or after step (f) encoding the memory with information that identifies the BB3 group added to each memory with matrix combination.

63. A tyrphostin analog compound produced by the process of claim 21.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZ
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07B 61/00, C07C 255/41, 323/32, C07D 213/40, B01J 19/00, C07D 317/58, C07C 255/60, C12Q 1/48, C07D 333/20, B01L 3/14, C07D 307/52, G06K 19/06

A3

(11) International Publication Number: WO 97/49653

(43) International Publication Date: 31 December 1997 (31.12.97)

(21) International Application Number: PCT/US97/11035

(22) International Filing Date: 24 June 1997 (24.06.97)

(30) Priority Data:

60/020,706	24 June 1996 (24.06.96)	US
08/711,426	5 September 1996 (05.09.96)	US
08/709,435	6 September 1996 (06.09.96)	US
08/723,423	30 September 1996 (30.09.96)	US

(71) Applicant *(for all designated States except US)*: IRORI [US/US]; Suite 100, 11025 North Torrey Pines Road, La Jolla, CA 92037 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: XIAO, Xiao-Yi [CN/US]; 4951 Riding Ridge Road, San Diego, CA 92130 (US). SHI, Shuhao [US/US]; 4178 Decoro Street #50, San Diego, CA 92122 (US). PARANDOOSH, Zahra [US/US]; 4626 Exbury Court, San Diego, CA 92130 (US). NOVA, Michael, P. [US/US]; 16428 La Gracia, Rancho Santa Fe, CA 92067 (US).

(74) Agent: SEIDMAN, Stephanie, L.; Brown Martin Haller & McClain, 1660 Union Street, San Diego, CA 92101-2926 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report: 26 February 1998 (26.02.98)

(54) Title: SOLID PHASE TYRPHOSTIN LIBRARY LINKED TO MATRICES WITH MEMORIES

(57) Abstract

Combinations, called matrices with memories, of matrix materials with memories that are remotely addressable or remotely programmable recording devices or are associated with imprinted symbology are provided. In particular, combinations of matrix materials, memories, and linked molecules that are tyrphostin analogs are provided. The matrix materials are those that are used in as supports in solid phase chemical and biochemical syntheses, immunoassays and hybridization reactions. The matrix materials may additionally include fluophors or other luminescent moieties to produce luminescing matrices with memories. The memories include electronic and optical storage media and also include optical memories, such as bar codes and other machine-readable codes. By virtue of this combination, molecules and biological particles, such as the tyrphostin compounds, that are in proximity or in physical contact with the matrix combination can be labeled by programming the memory with identifying information and can be identified by retrieving the stored information. The tyrphostin compounds, as well as methods of preparing the compounds, are also provided.

INTERNATIONAL SEARCH REPORT

Intern: al Application No
ᐣCT/US 97/11035

A. CLASSIFICATION OF SUBJECT M R
IPC 6 C07B61/00 B01J19/00 C07C255/41 C07D317/58 C07C323/32
C07C255/60 C07D333/20 C07D307/52 C07D213/40 C12Q1/48
B01L3/14 G06K19/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07B C07C C07D B01L B01J C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 122, no. 9, 27 February 1995 Columbus, Ohio, US; abstract no. 105410z, XP002051044 see abstract; RN 149551-25-7, 149551-28-0, 149551-40-6, 149551-41-7, 149551-50-8, 149551-53-1, 149551-58-6	2-8,63
A	see abstract	21,28
	& JP 06 247 850 A (SUNTORY LTD) 6 September 1994	

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
30 December 1997	15.01.98
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Van Amsterdam, L

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	N. OSHEROV ET AL: J. BIOL. CHEM., vol. 268, no. 15, 1993, pages 11134-11142, XP002051039 see page 11136, table II, AG933	2,4-8,63
A	see page 11135, table I, AG490	2

X	WO 95 19169 A (SUGEN INC ET AL) 20 July 1995 see figure 1K, compound P25	2,4-8,63
A	see page 133, lines 15-25	21,28

X	EP 0 537 742 A (MITSUBISHI KASEI CORP) 21 April 1993 see table 1, compounds 30, 35, 36, 113; claims 1-3	2-7,63
A	see page 21, lines 28-54; page 23, line 52 - page 24, line 27	21,28, 52-54, 56,57

X	WO 95 24190 A (SUGEN INC ET AL) 14 September 1995 see table I and page 78, compound M23	2,4-7,63
A	see page 78, lines 11-24	21,28

X	CHEMICAL ABSTRACTS, vol. 110, no. 1, 2 January 1989 Columbus, Ohio, US; abstract no. 7874m, XP002051045 see abstract; RN 117906-51-1, 117906-65-7 & JP 63 154 663 A (KANEGAFUCHI CHEMICAL INDUSTRY CO LTD) 27 June 1988	2,4-7,63

X	J. DUQUE ET AL: REV. CENIC, CIENC. QUIM., vol. 27, no. 1-2-3, 1996, pages 25-29, XP002051040 see the whole document	2,4,7,63

X	O. DURRUTHY ET AL: ACTA CRYSTALLOGR., SECT. C: CRYST. STRUCT. COMMUN., vol. C49, no. 3, 1993, pages 558-559, XP002051041 see the whole document	2,4,7,63
	---	
2 X	A.D. DELANEY ET AL: CAN. J. CHEM., vol. 47, no. 17, 1969, pages 3273-3277, XP002051042 see table II, entries 2-8, 14-21, 25-32	2,3,63

	-/--	

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/11035

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 109, no. 21, 21 November 1988 Columbus, Ohio, US; abstract no. 189995m, XP002051046 see abstract; RN 117133-11-6 & JP 63 150 223 A (KANEGAFUCHI CHEMICAL INDUSTRY CO LTD) 22 June 1988 ---	2,3,63
X	CHEMICAL ABSTRACTS, vol. 106, no. 3, 19 January 1987 Columbus, Ohio, US; abstract no. 18560z, XP002051047 see abstract; RN 105956-03-4 & JP 61 189 272 A (TAISHO PHARMACEUTICAL CO LTD) 22 August 1986 ---	2,3,63
X	I. KATSUMI ET AL: CHEM. PHARM. BULL., vol. 34, no. 4, 1986, pages 1619-1627, XP002051043 see table I, compound 30	2,3,63
A	see page 1626, lines 2-9 ---	21,28
X	CHEMICAL ABSTRACTS, vol. 120, no. 13, 28 March 1994 Columbus, Ohio, US; abstract no. 163847s, XP002051048 see abstract; RN 153139-75-4, 153139-76-5, 153139-77-6 & R. JINDAL ET AL: J. INDIAN CHEM. SOC., vol. 70, no. 1, 1993, pages 80-81, ---	2,3,63
X	CHEMICAL ABSTRACTS, vol. 112, no. 8, 19 February 1990 Columbus, Ohio, US; abstract no. 66629z, XP002051049 see abstract; RN 124348-10-3 & JP 01 179 041 A (KONICA CO) 17 July 1989 ---	2,3,63
X	CHEMICAL ABSTRACTS, vol. 101, no. 12, 17 September 1984 Columbus, Ohio, US; abstract no. 101209, XP002051050 see abstract; RN 91470-80-3 & JP 59 007 956 A (CANON KK) 17 January 1984 --- -/--	2,3,63

2

INTERNATIONAL SEARCH REPORT

Intern   al Application No
P   IS 97/11035

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 89, no. 17, 23 October 1978 Columbus, Ohio, US; abstract no. 146729d, XP002051051 see abstract; RN 23795-36-0 & A. LORENTE ET AL: AFINIDAD, vol. 35, no. 354, 1978, pages 138-140, ---	2,3,63
X	WO 95 26341 A (PHARMACIA SPA) 5 October 1995 see page 25, lines 17-24; page 26, lines 10-13; page 27, lines 1, 7-9, 21; page 28, lines 2-3, 9-10, 15, 18, 24, 27; page 29, lines 4-5, 16-17; page 30, lines 2, 15-19; page 31, lines 5-11; claims 1-4	2,63
A	see page 10, line 12 - page 15, line 18; page 18 ---	21,28, 52-54, 56,57
A	WO 93 06121 A (AFFYMAX TECHNOLOGIES NV) 1 April 1993 see page 7, lines 21-33; page 20, line 19 - page 21, line 4 -----	11-20

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna. .ial Application No
CT/US 97/11035

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9519169 A	20-07-95	AU 1563395 A	01-08-95
		CA 2180658 A	20-07-95
		CN 1128496 A	07-08-96
		EP 0804191 A	05-11-97
		US 5610173 A	11-03-97
EP 537742 A	21-04-93	CA 2080554 A	16-04-93
		DE 69212948 D	26-09-96
		DE 69212948 T	13-02-97
		ES 2093753 T	01-01-97
		US 5514711 A	07-05-96
		JP 5301838 A	16-11-93
WO 9524190 A	14-09-95	AU 2096895 A	25-09-95
WO 9526341 A	05-10-95	AU 1849895 A	17-10-95
		CN 1125942 A	03-07-96
		EP 0700388 A	13-03-96
		FI 955661 A	19-01-96
		HU 73811 A	30-09-96
		JP 8511562 T	03-12-96
		NO 954692 A	20-11-95
		PL 311745 A	18-03-96
		US 5652250 A	29-07-97
		ZA 9502423 A	19-12-95
WO 9306121 A	01-04-93	AT 148889 T	15-02-97
		AU 669489 B	13-06-96
		AU 2661992 A	27-04-93
		CA 2118806 A	01-04-93
		DE 69217497 D	27-03-97
		DE 69217497 T	12-06-97
		EP 0604552 A	06-07-94
		EP 0773227 A	14-05-97
		ES 2097925 T	16-04-97
		US 5639603 A	17-06-97

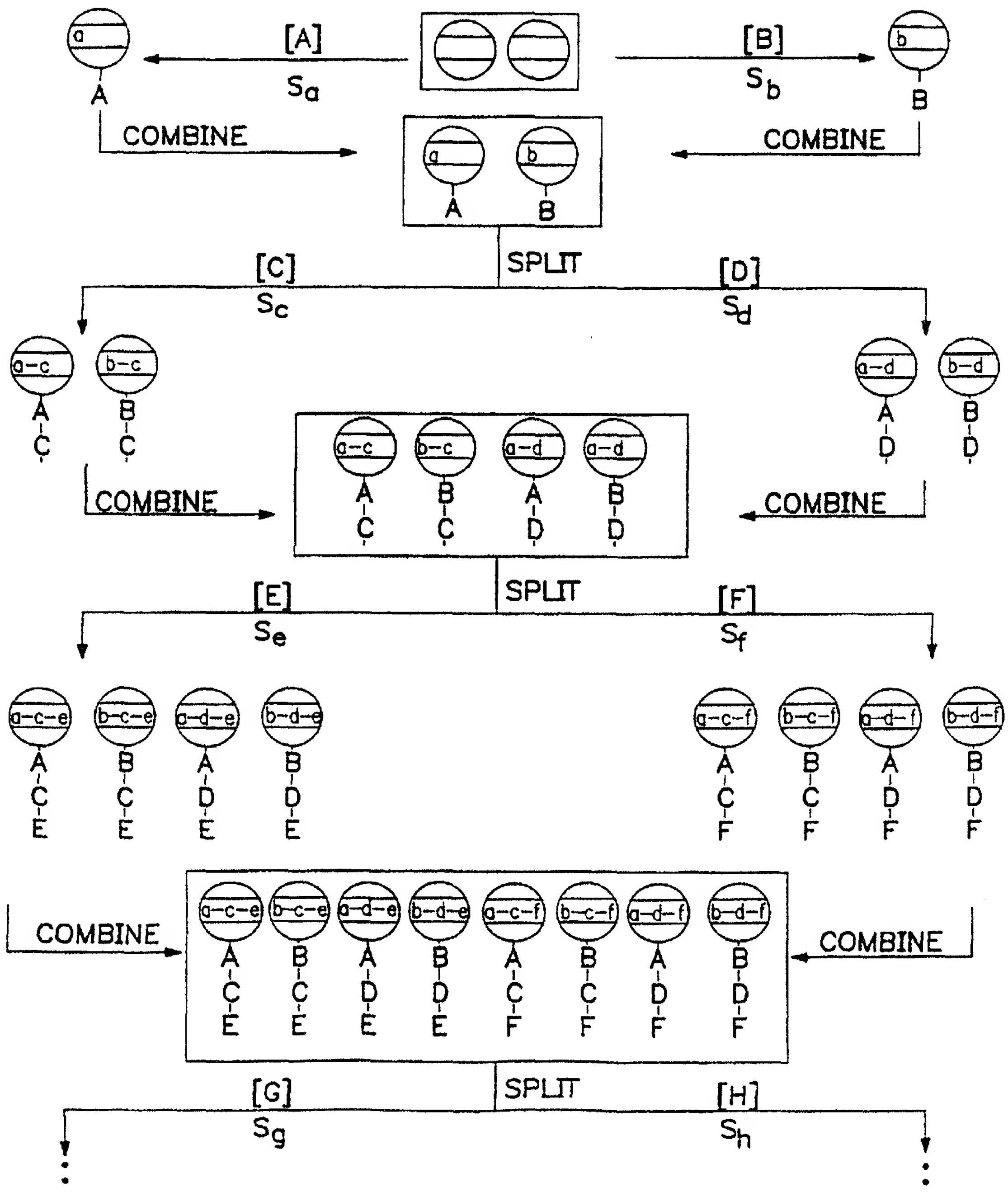

[A]
S_a
[B]
S_b
a
A
b
B
COMBINE
COMBINE
SPLIT
[C]
S_c
[D]
S_d
a–c
b–c
a–d
b–d
COMBINE
COMBINE
SPLIT
[E]
S_e
[F]
S_f
a–c–e
b–c–e
a–d–e
b–d–e
a–c–f
b–c–f
a–d–f
b–d–f
COMBINE
COMBINE
SPLIT
[G]
S_g
[H]
S_h

FIG. 1

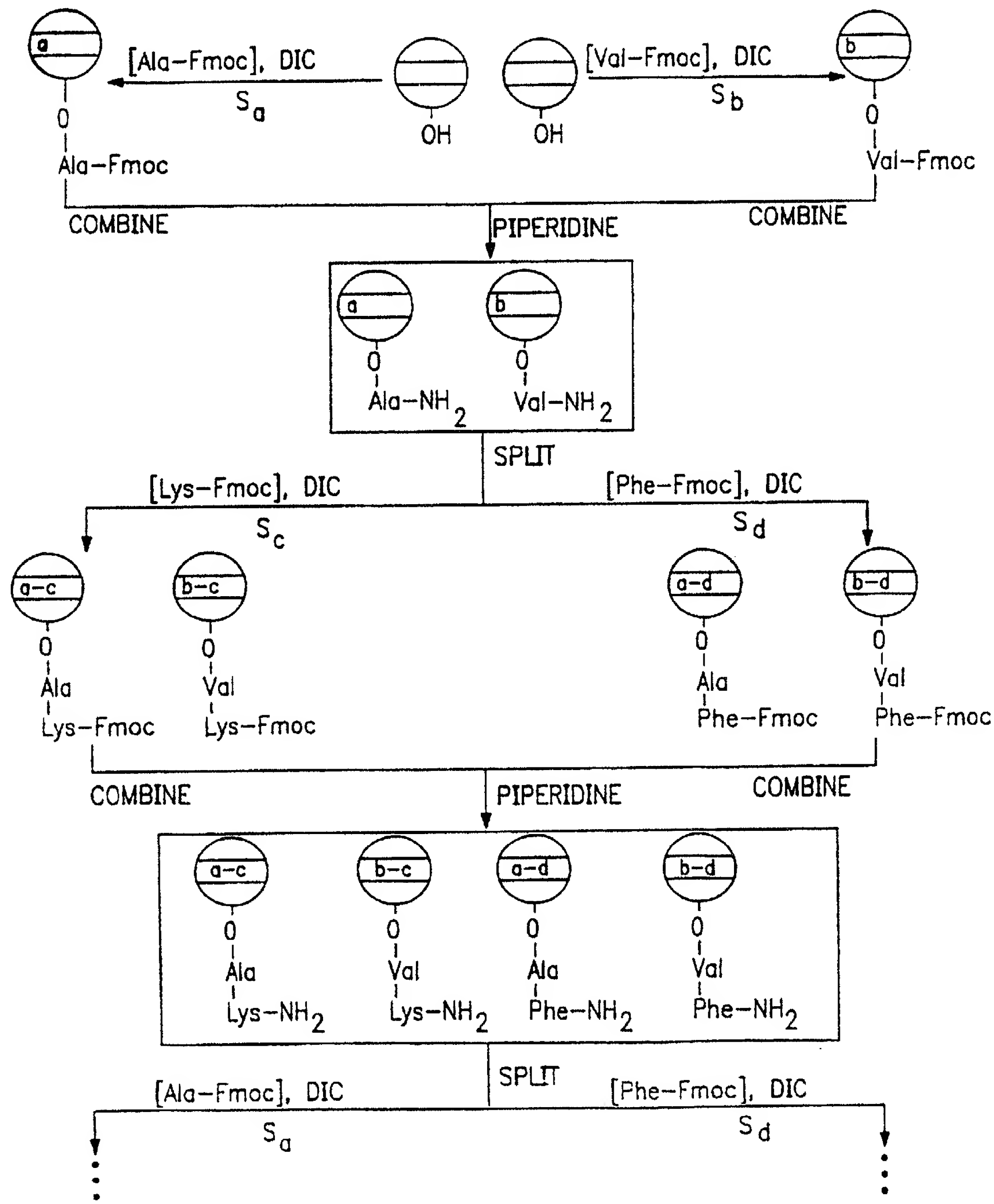

[Ala-Fmoc], DIC
Sa
[Val-Fmoc], DIC
Sb
a
b
OH
OH
O
Ala-Fmoc
Val-Fmoc
COMBINE
PIPERIDINE
COMBINE
Ala-NH2
Val-NH2
SPLIT
[Lys-Fmoc], DIC
Sc
[Phe-Fmoc], DIC
Sd
a-c
b-c
a-d
b-d
Ala
Val
Lys-Fmoc
Phe-Fmoc
COMBINE
PIPERIDINE
COMBINE
Lys-NH2
Phe-NH2
SPLIT
[Ala-Fmoc], DIC
Sa
[Phe-Fmoc], DIC
Sd

FIG. 2

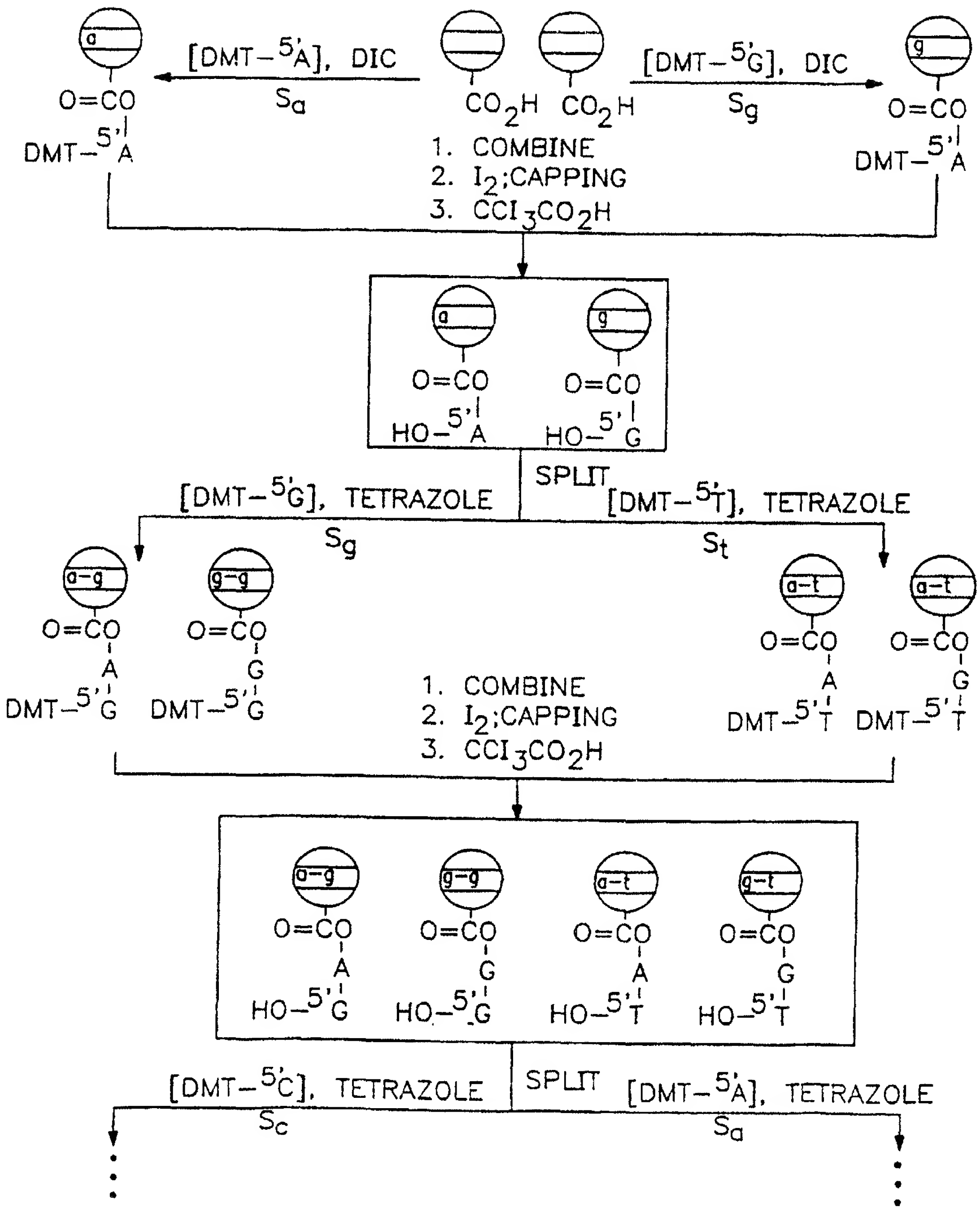

[DMT–5'A], DIC
Sa
[DMT–5'G], DIC
Sg
O=CO
DMT–5'A
CO2H CO2H
1. COMBINE
2. I2;CAPPING
3. CCl3CO2H
HO–5'A
HO–5'G
SPLIT
[DMT–5'G], TETRAZOLE
Sg
[DMT–5'T], TETRAZOLE
St
DMT–5'G
DMT–5'T
HO–5'G
HO–5'T
[DMT–5'C], TETRAZOLE
Sc
[DMT–5'A], TETRAZOLE
Sa

FIG. 3

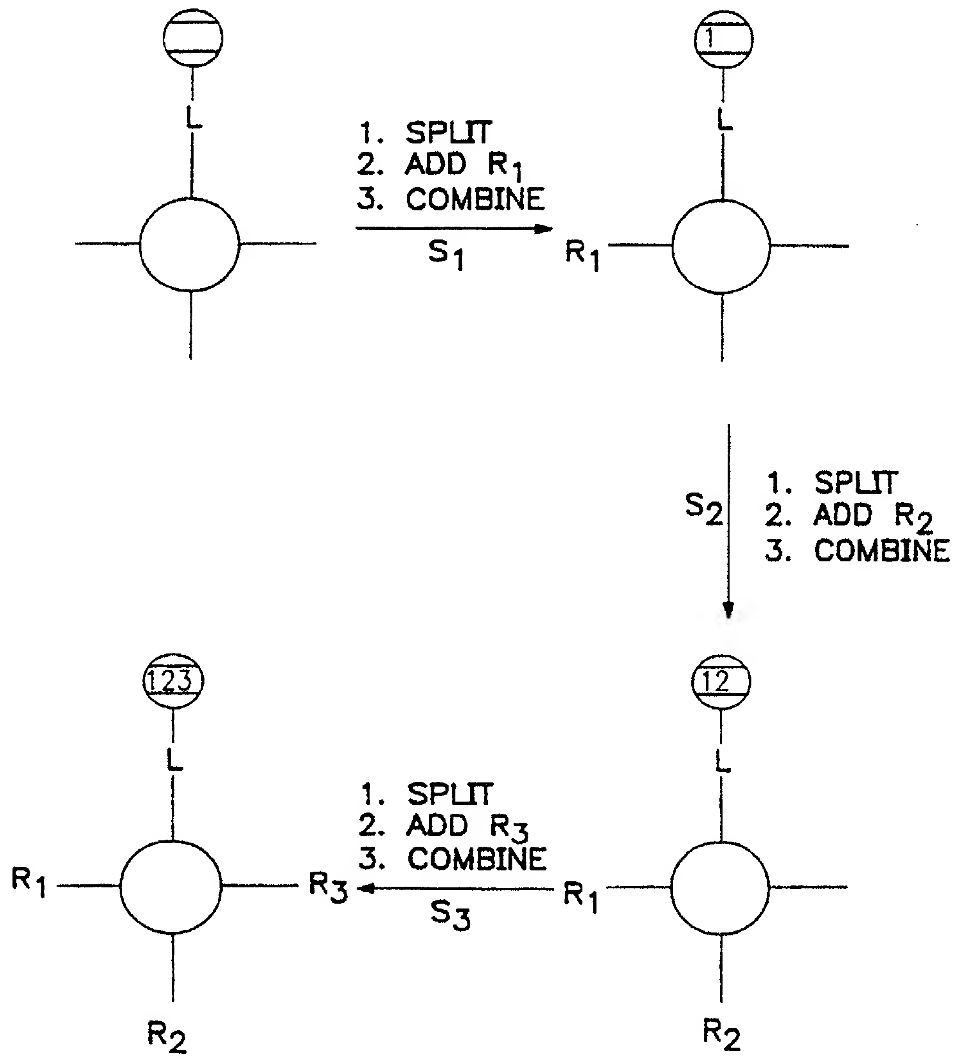

L
1. SPLIT
2. ADD R_1
3. COMBINE
S_1
R_1
1
L
S_2
1. SPLIT
2. ADD R_2
3. COMBINE
123
L
12
L
1. SPLIT
2. ADD R_3
3. COMBINE
R_1
R_3
S_3
R_1
R_2
R_2

FIG. 4

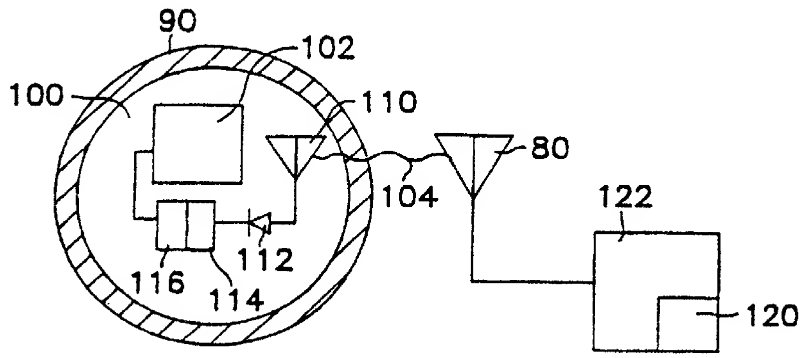

90
102
100
110
80
104
122
112
116
114
120

FIG. 5

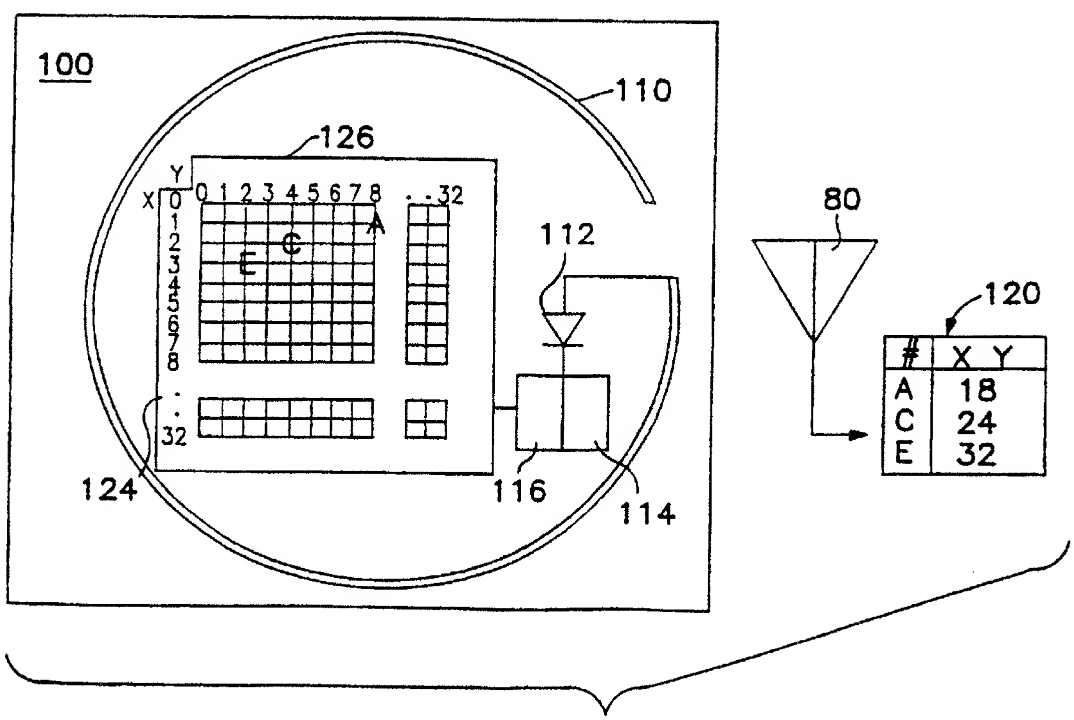

100
110
126
Y
X
0 1 2 3 4 5 6 7 8 . . 32
0
1
2
3
4
5
6
7
8
.
.
32
A
C
E
112
80
120
X Y
A 18
C 24
E 32
124
116
114

FIG. 6

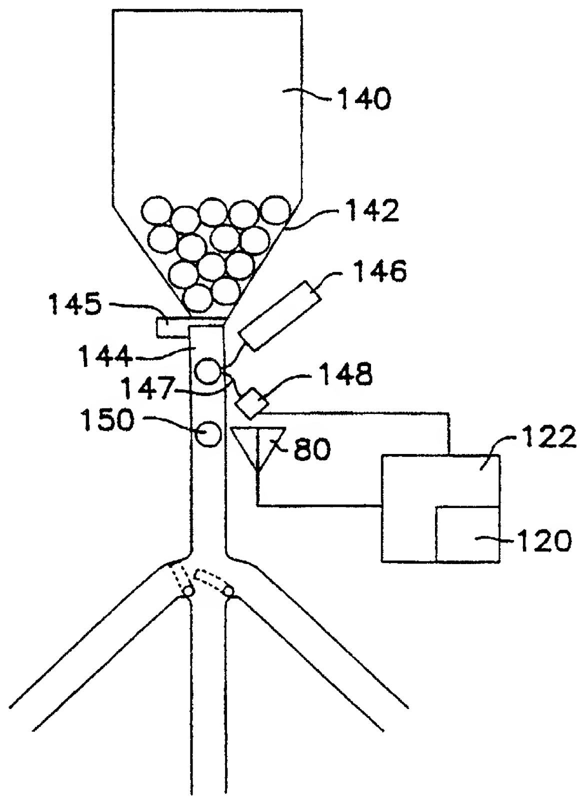


FIG. 7

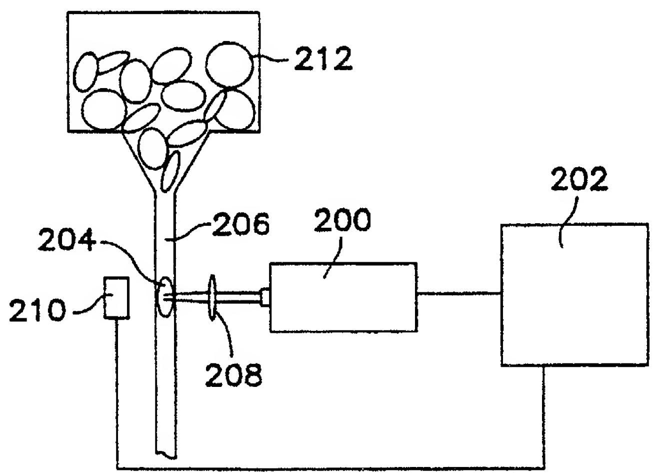


FIG. 8

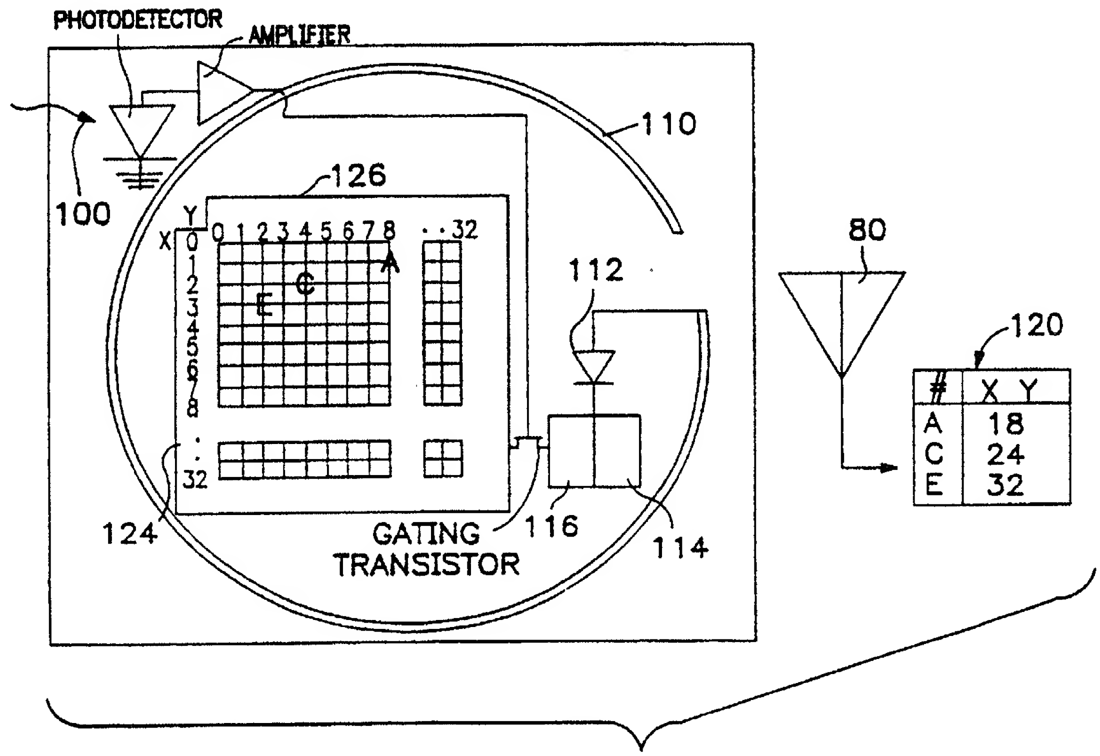

PHOTODETECTOR
AMPLIFIER
100
110
126
Y
X
0 1 2 3 4 5 6 7 8 . . 32
0
1
2
3
4
5
6
7
8
.
.
32
A
E
C
112
80
120
X Y
A 18
C 24
E 32
124
GATING TRANSISTOR
116
114

FIG. 9

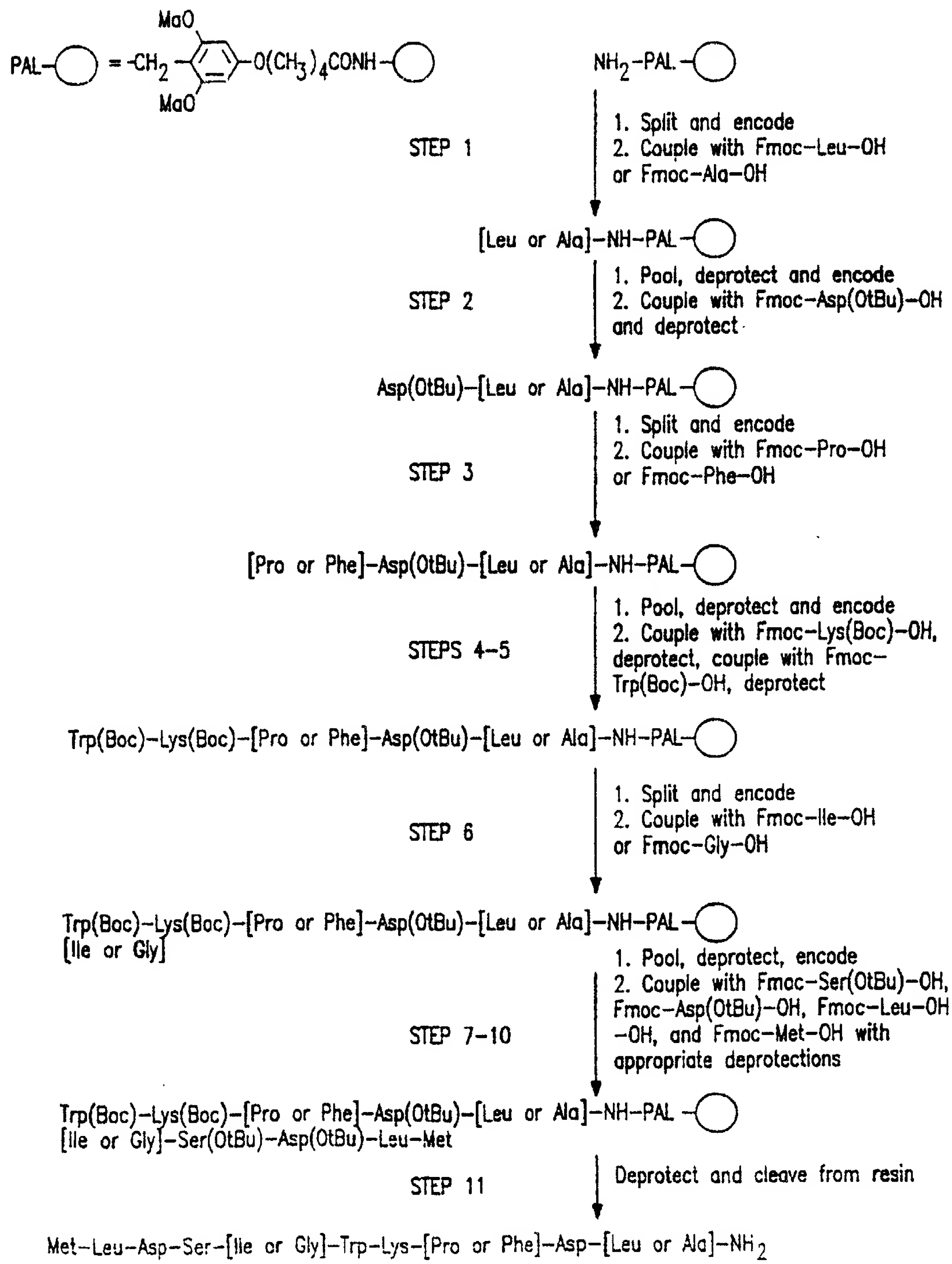

MeO
PAL-◯ = -CH2-⟨⟩-O(CH3)4CONH-◯
MeO
NH2-PAL-◯
STEP 1
1. Split and encode
2. Couple with Fmoc-Leu-OH or Fmoc-Ala-OH
[Leu or Ala]-NH-PAL-◯
STEP 2
1. Pool, deprotect and encode
2. Couple with Fmoc-Asp(OtBu)-OH and deprotect
Asp(OtBu)-[Leu or Ala]-NH-PAL-◯
STEP 3
1. Split and encode
2. Couple with Fmoc-Pro-OH or Fmoc-Phe-OH
[Pro or Phe]-Asp(OtBu)-[Leu or Ala]-NH-PAL-◯
STEPS 4-5
1. Pool, deprotect and encode
2. Couple with Fmoc-Lys(Boc)-OH, deprotect, couple with Fmoc-Trp(Boc)-OH, deprotect
Trp(Boc)-Lys(Boc)-[Pro or Phe]-Asp(OtBu)-[Leu or Ala]-NH-PAL-◯
STEP 6
1. Split and encode
2. Couple with Fmoc-Ile-OH or Fmoc-Gly-OH
Trp(Boc)-Lys(Boc)-[Pro or Phe]-Asp(OtBu)-[Leu or Ala]-NH-PAL-◯
[Ile or Gly]
STEP 7-10
1. Pool, deprotect, encode
2. Couple with Fmoc-Ser(OtBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Leu-OH -OH, and Fmoc-Met-OH with appropriate deprotections
Trp(Boc)-Lys(Boc)-[Pro or Phe]-Asp(OtBu)-[Leu or Ala]-NH-PAL-◯
[Ile or Gly]-Ser(OtBu)-Asp(OtBu)-Leu-Met
STEP 11
Deprotect and cleave from resin
Met-Leu-Asp-Ser-[Ile or Gly]-Trp-Lys-[Pro or Phe]-Asp-[Leu or Ala]-NH2

FIG. 10

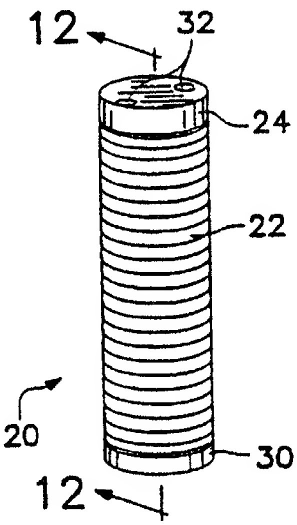


FIG. 11

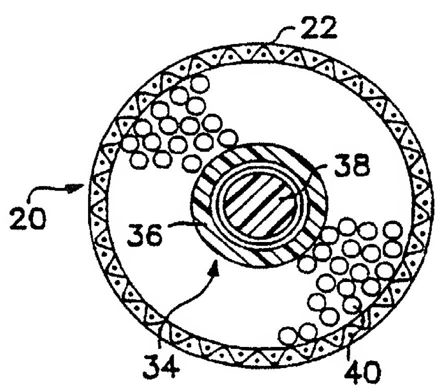


FIG. 13

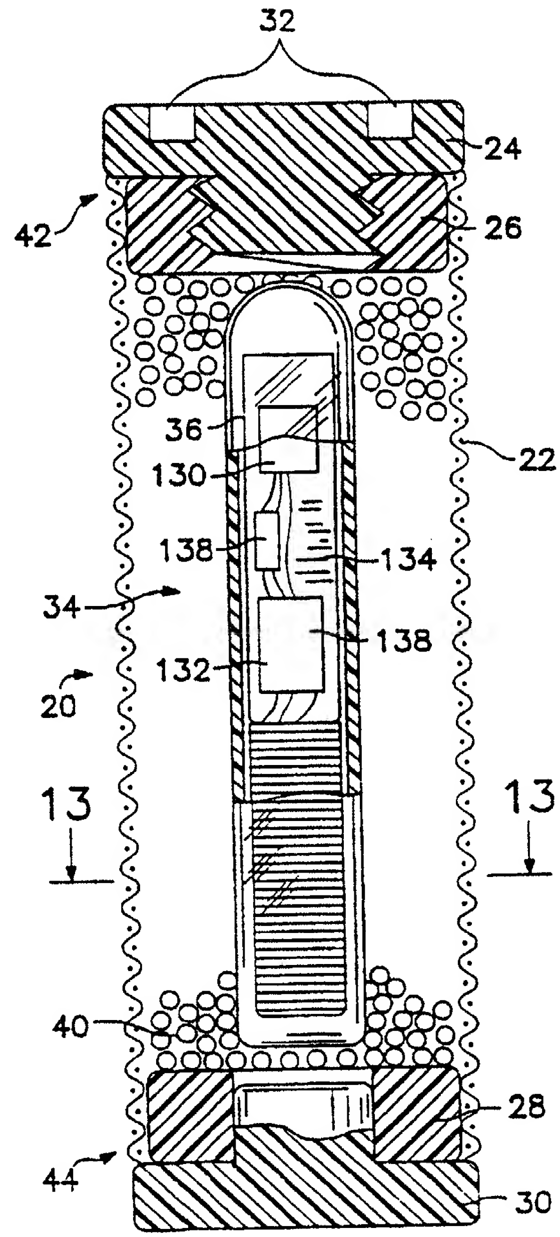


FIG. 12

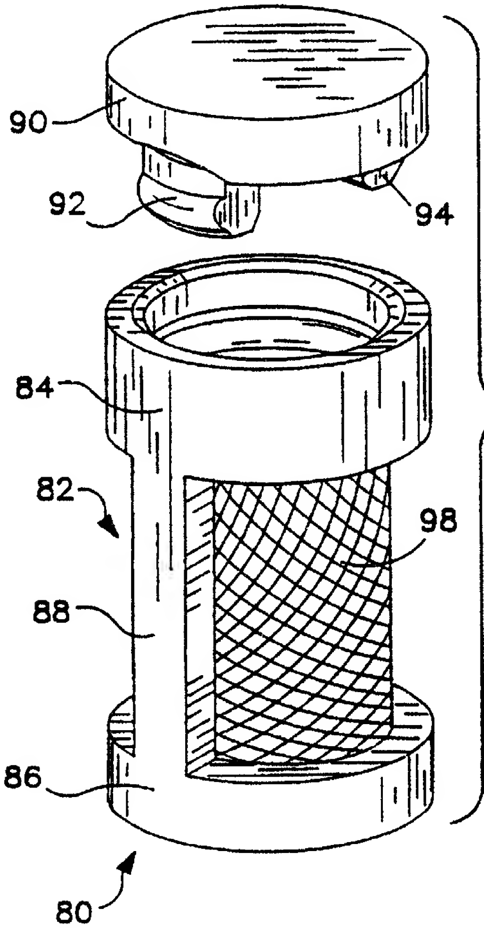


FIG. 14

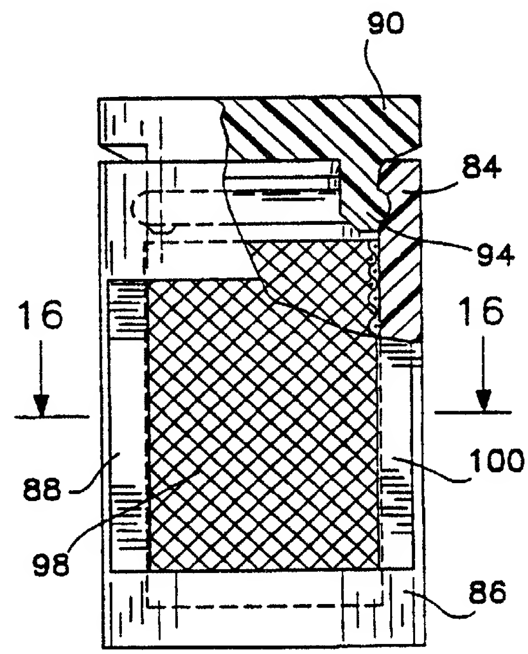


FIG. 15

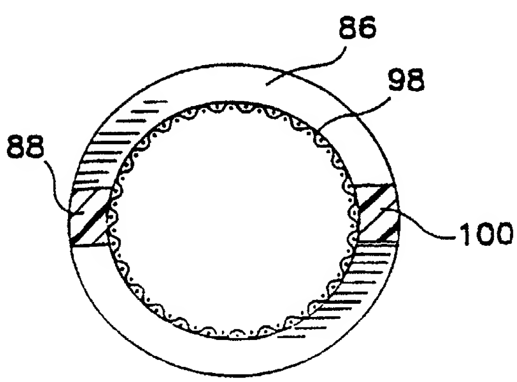


FIG. 16

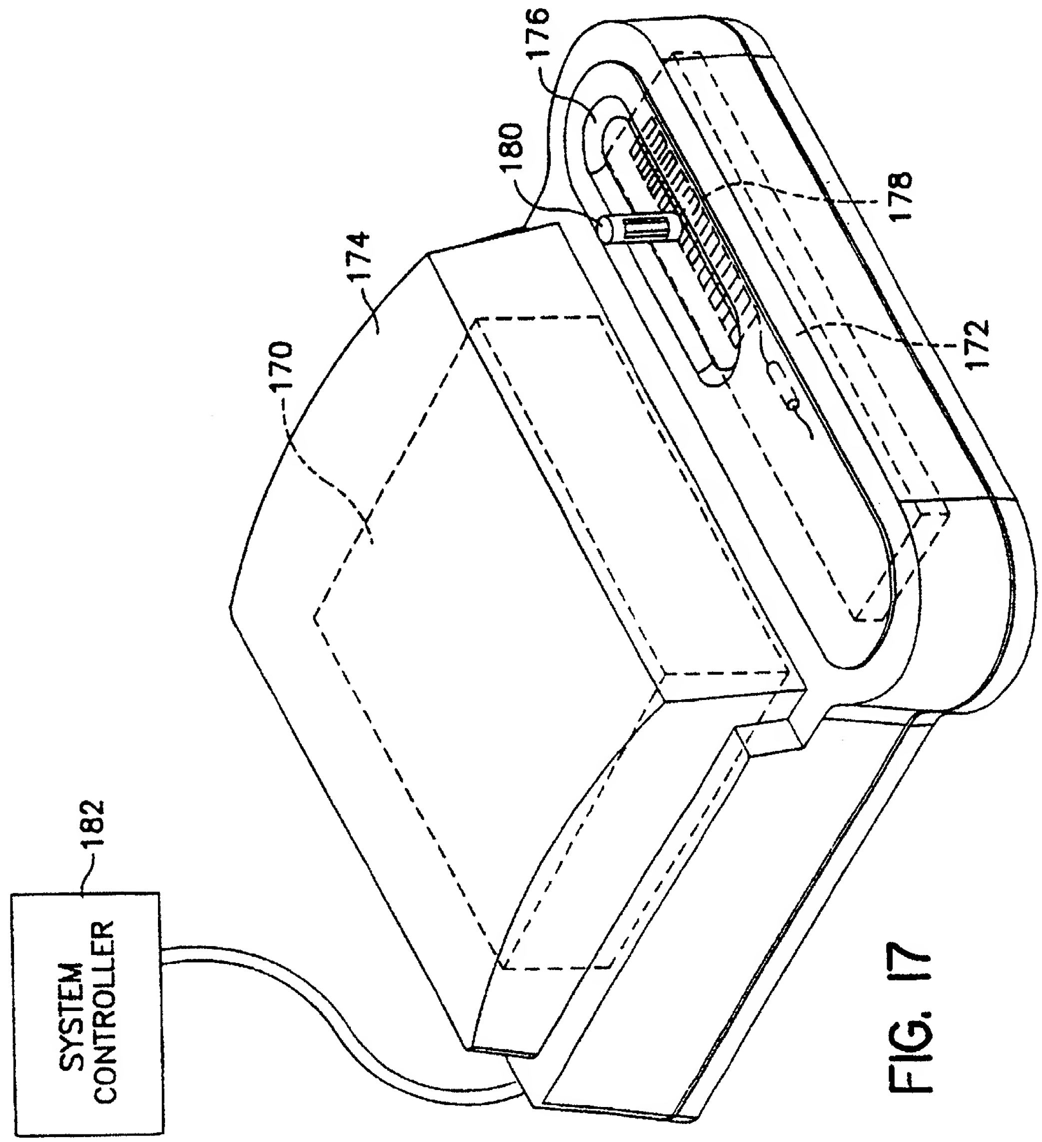


FIG. 17

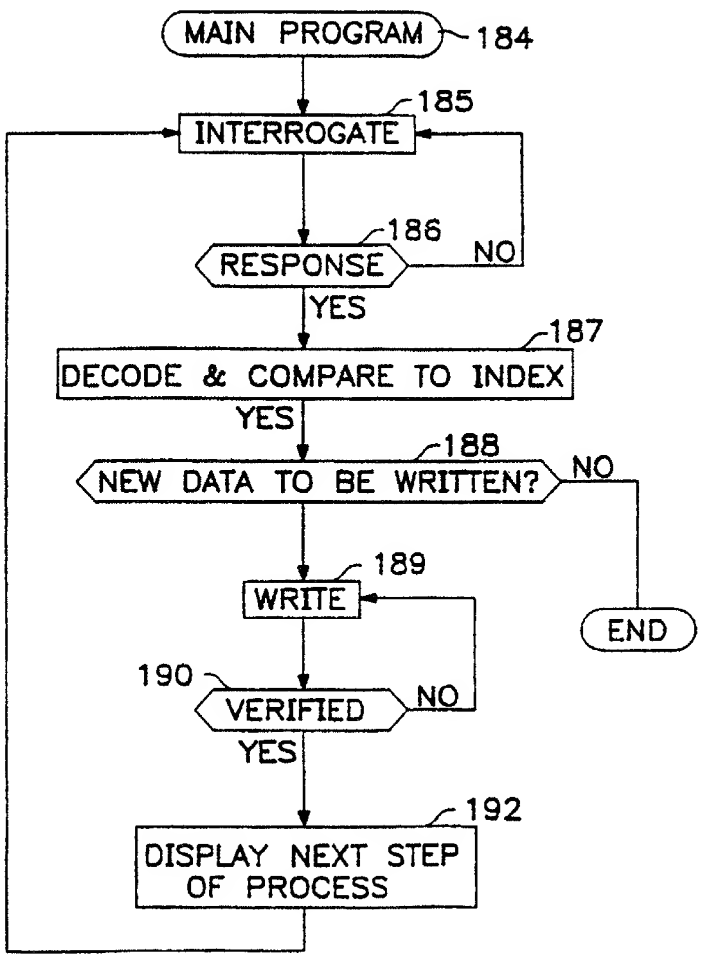

MAIN PROGRAM
184
185
INTERROGATE
186
RESPONSE
NO
YES
187
DECODE & COMPARE TO INDEX
YES
188
NEW DATA TO BE WRITTEN?
NO
189
WRITE
END
190
VERIFIED
NO
YES
192
DISPLAY NEXT STEP OF PROCESS

FIG. 18

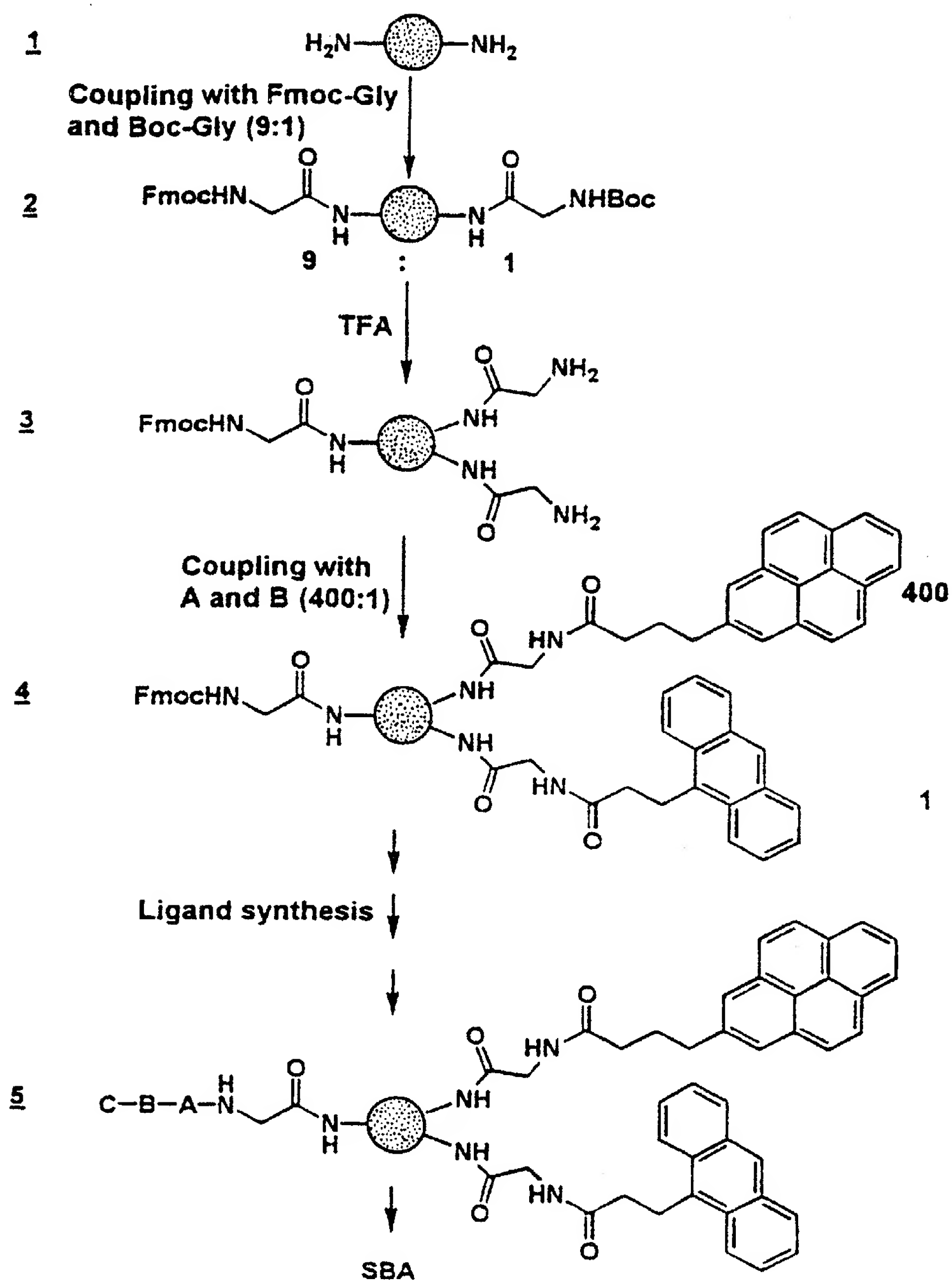

1
H2N NH2
Coupling with Fmoc-Gly and Boc-Gly (9:1)
2
FmocHN NHBoc
9 : 1
TFA
3
FmocHN NH2 NH2
Coupling with A and B (400:1)
400
4
FmocHN HN NH
1
Ligand synthesis
5
C—B—A—N
SBA

FIG. 19

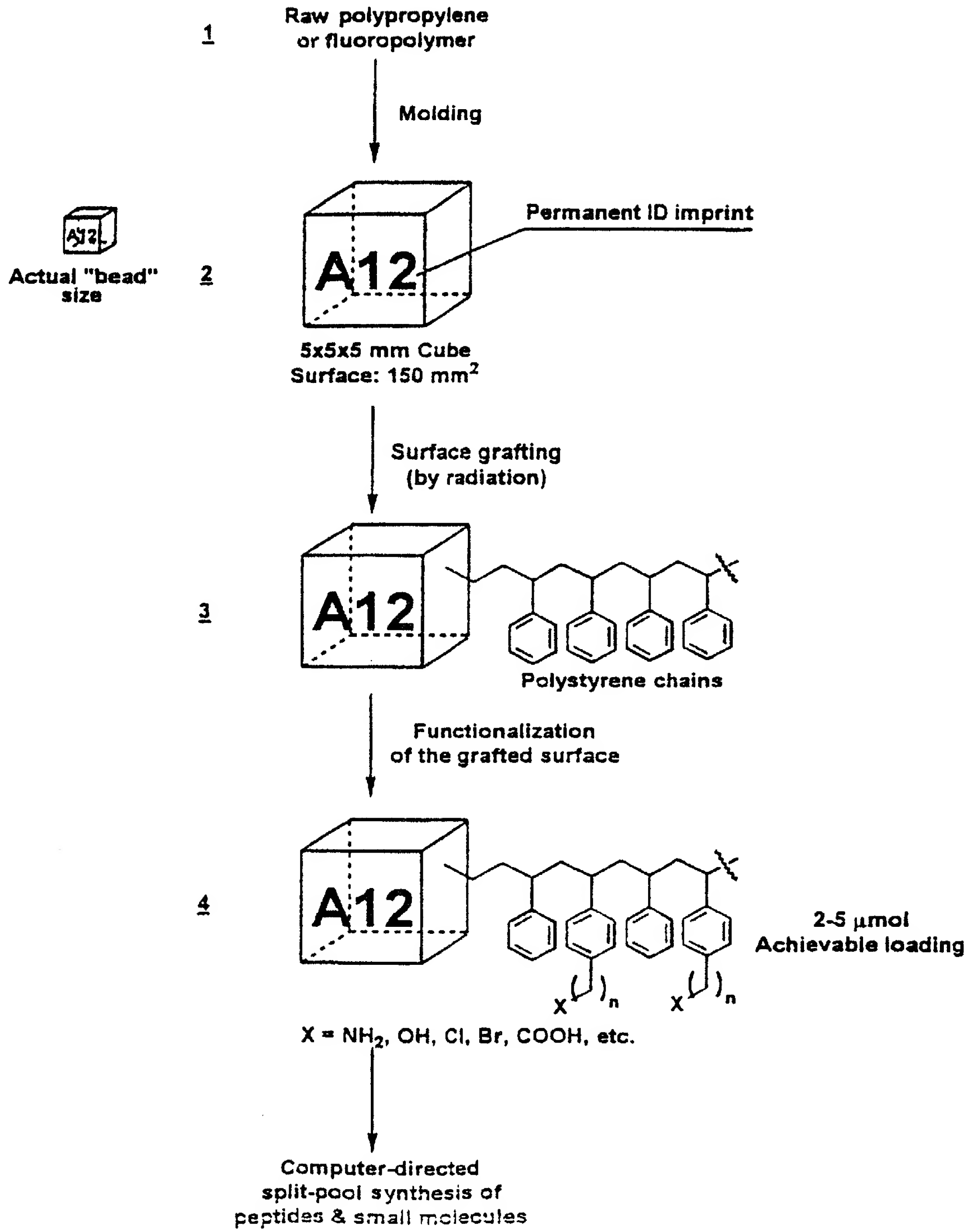

1
Raw polypropylene or fluoropolymer
Molding
Actual "bead" size
2
A12
Permanent ID imprint
5x5x5 mm Cube
Surface: 150 mm2
Surface grafting (by radiation)
3
A12
Polystyrene chains
Functionalization of the grafted surface
4
A12
2-5 µmol
Achievable loading
X
n
X = NH2, OH, Cl, Br, COOH, etc.
Computer-directed split-pool synthesis of peptides & small molecules

FIG. 20

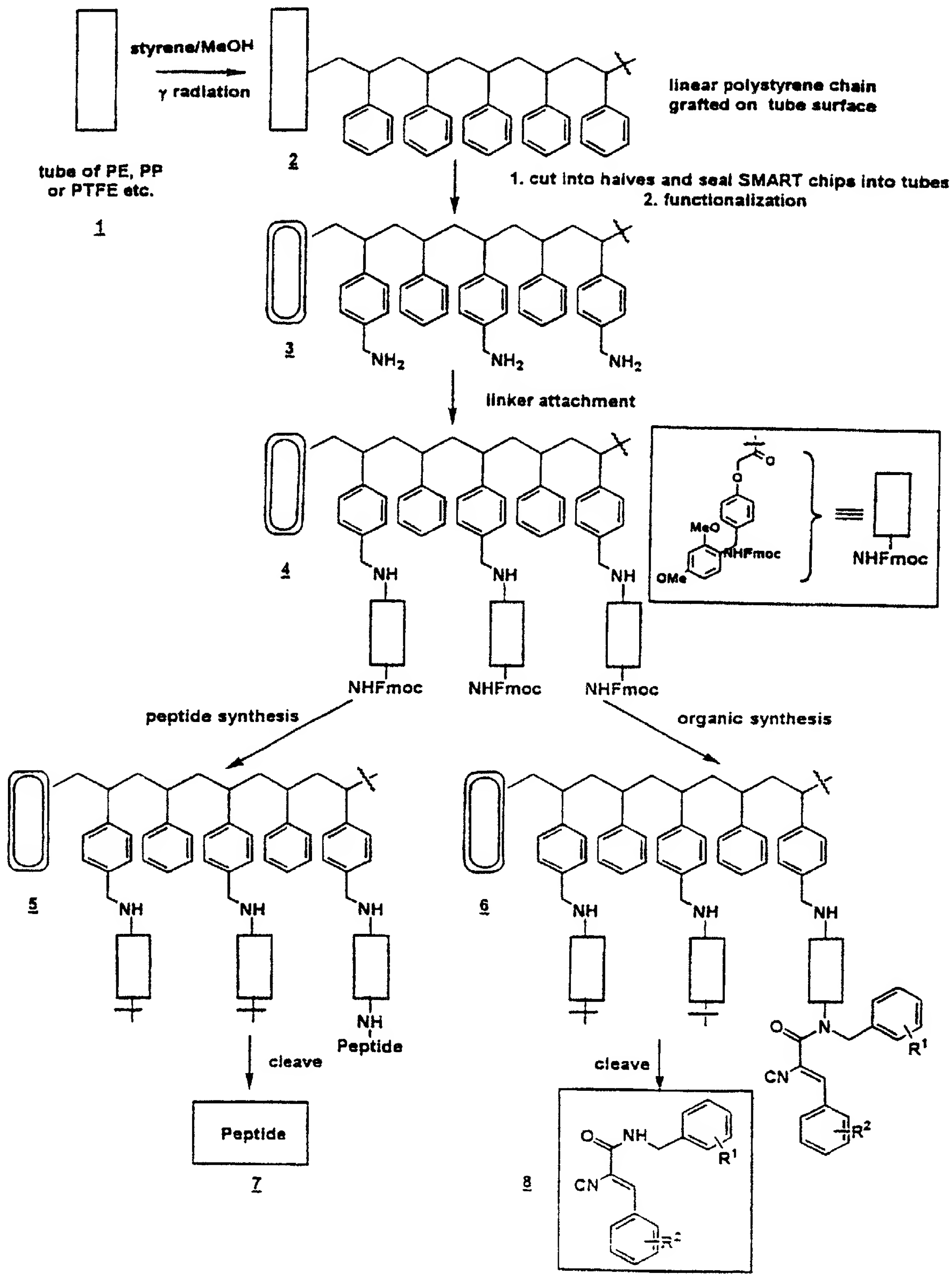

styrene/MeOH
γ radiation
linear polystyrene chain grafted on tube surface
tube of PE, PP or PTFE etc.
1
2
1. cut into halves and seal SMART chips into tubes
2. functionalization
3
NH2
linker attachment
4
NH
NHFmoc
MeO
OMe
peptide synthesis
organic synthesis
5
6
Peptide
cleave
7
8
CN
R1
R2

FIG. 21

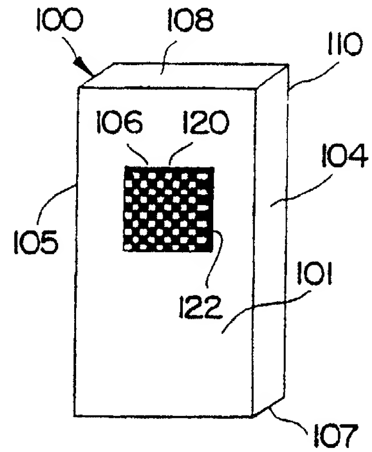


FIG. 22

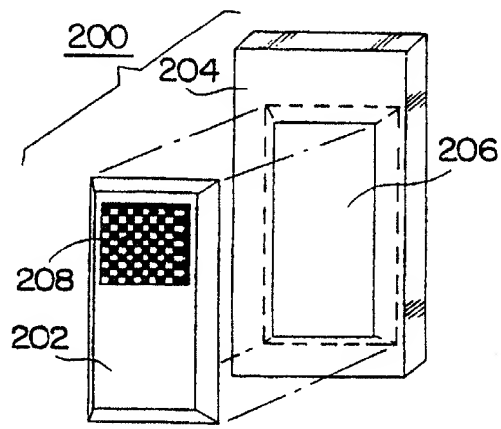


FIG. 23

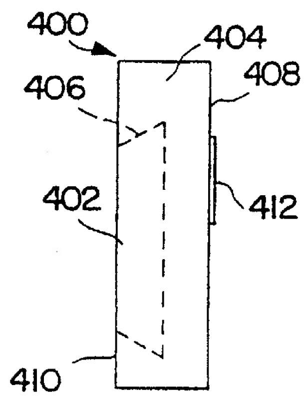


FIG. 25

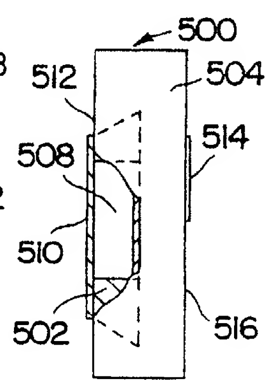


FIG. 26

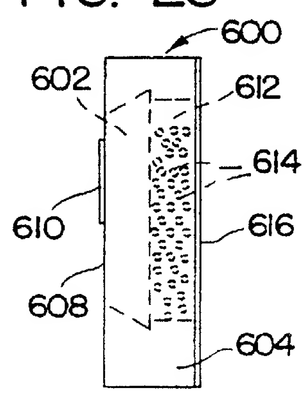


FIG. 27

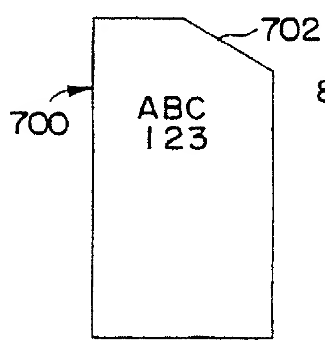


FIG. 28

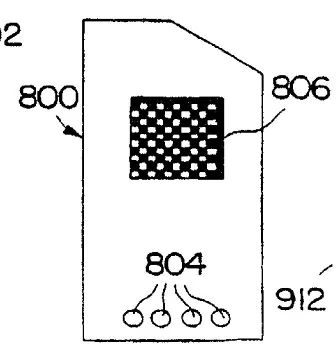


FIG. 29

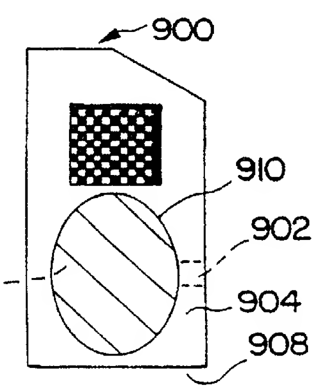


FIG. 30

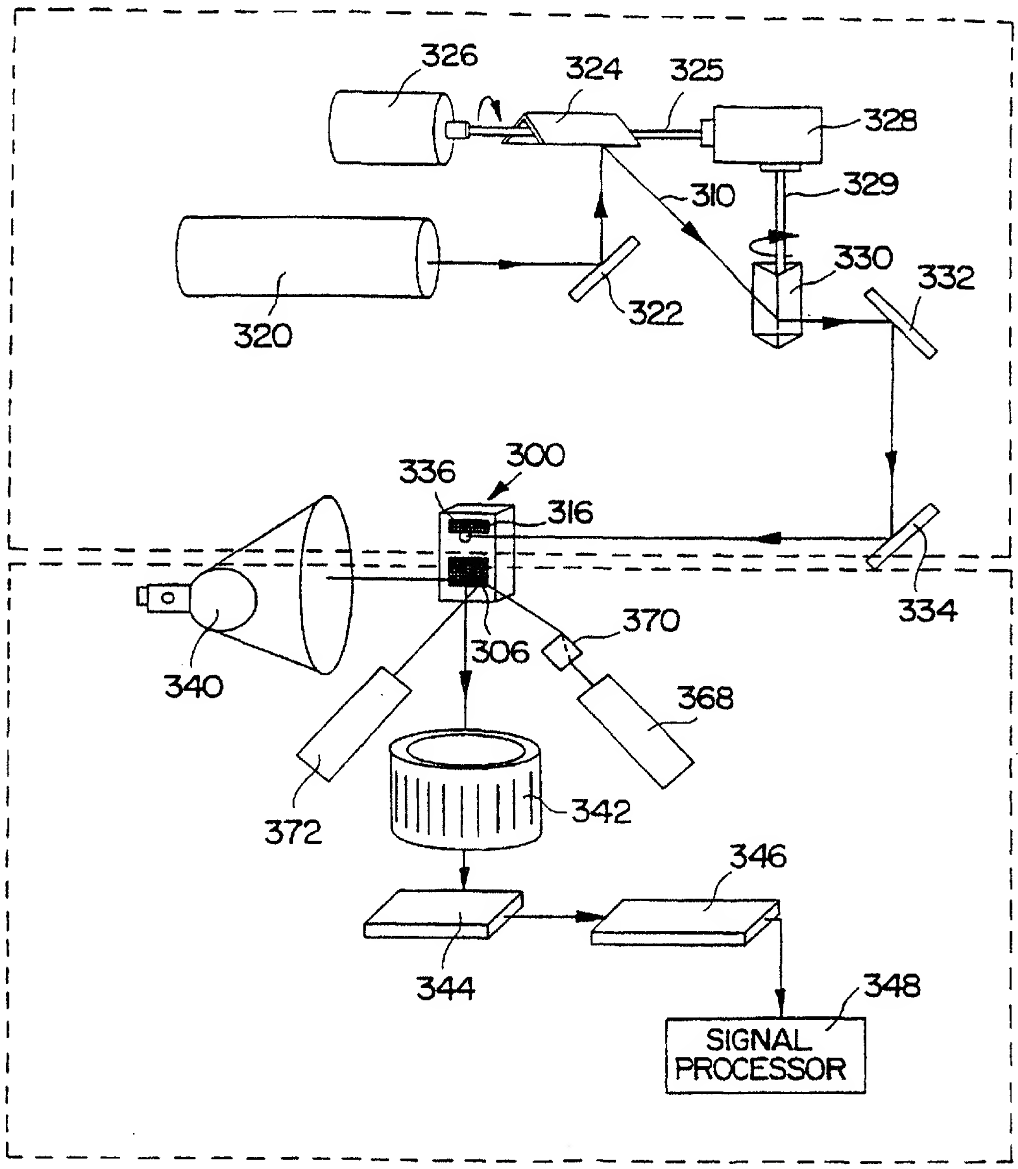

326
324
325
328
310
329
320
322
330
332
336
300
316
334
340
306
370
368
372
342
346
344
348
SIGNAL PROCESSOR

FIG. 24

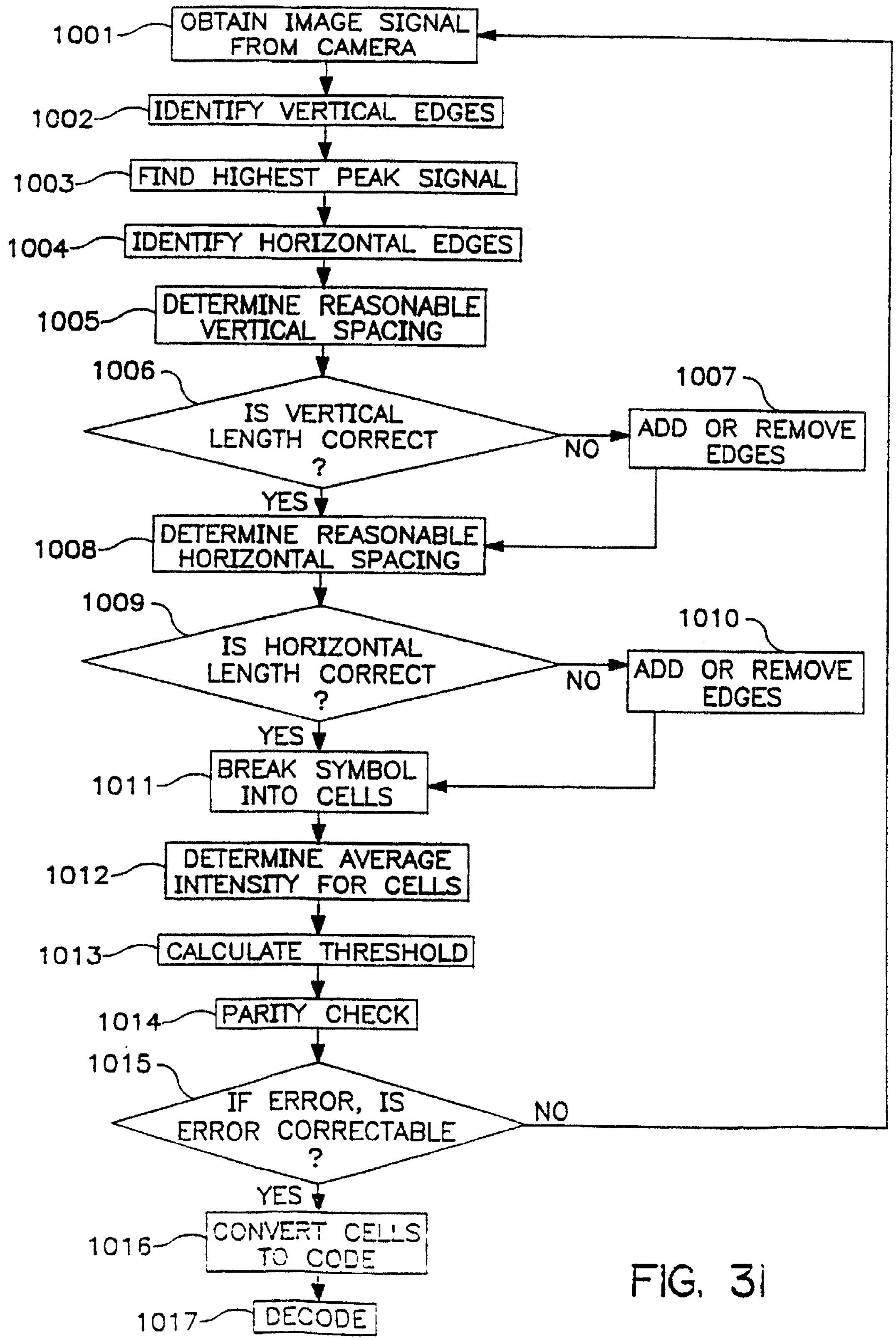

1001
OBTAIN IMAGE SIGNAL FROM CAMERA
1002
IDENTIFY VERTICAL EDGES
1003
FIND HIGHEST PEAK SIGNAL
1004
IDENTIFY HORIZONTAL EDGES
1005
DETERMINE REASONABLE VERTICAL SPACING
1006
IS VERTICAL LENGTH CORRECT ?
NO
1007
ADD OR REMOVE EDGES
YES
1008
DETERMINE REASONABLE HORIZONTAL SPACING
1009
IS HORIZONTAL LENGTH CORRECT ?
NO
1010
ADD OR REMOVE EDGES
YES
1011
BREAK SYMBOL INTO CELLS
1012
DETERMINE AVERAGE INTENSITY FOR CELLS
1013
CALCULATE THRESHOLD
1014
PARITY CHECK
1015
IF ERROR, IS ERROR CORRECTABLE ?
NO
YES
1016
CONVERT CELLS TO CODE
1017
DECODE

FIG. 3I

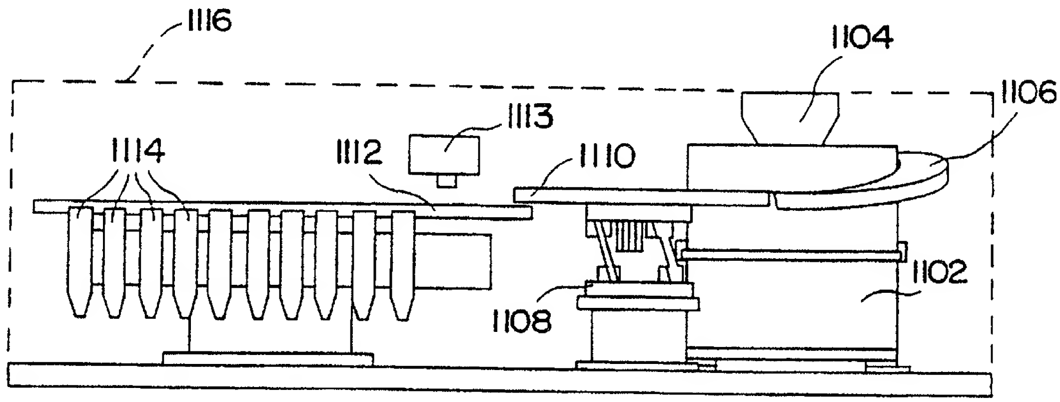


FIG. 32

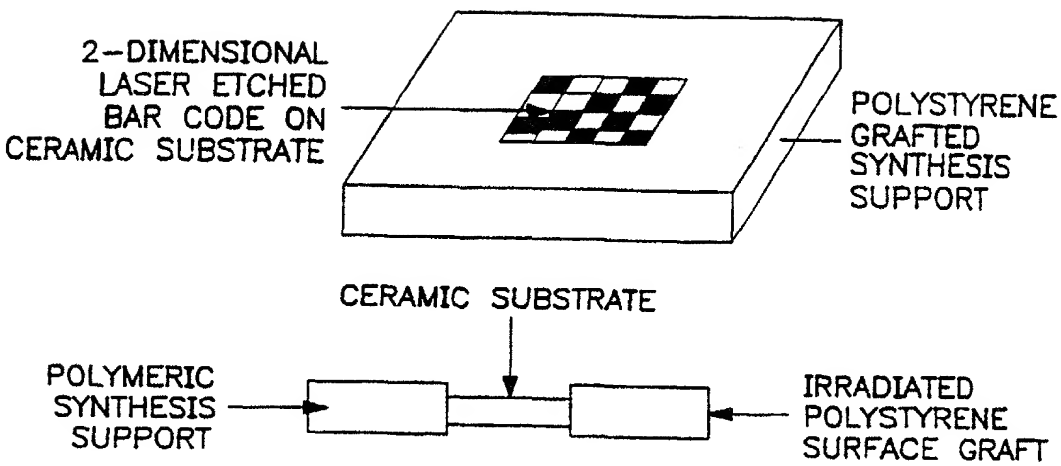


FIG. 33A

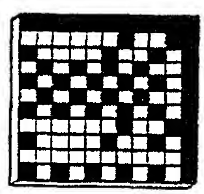
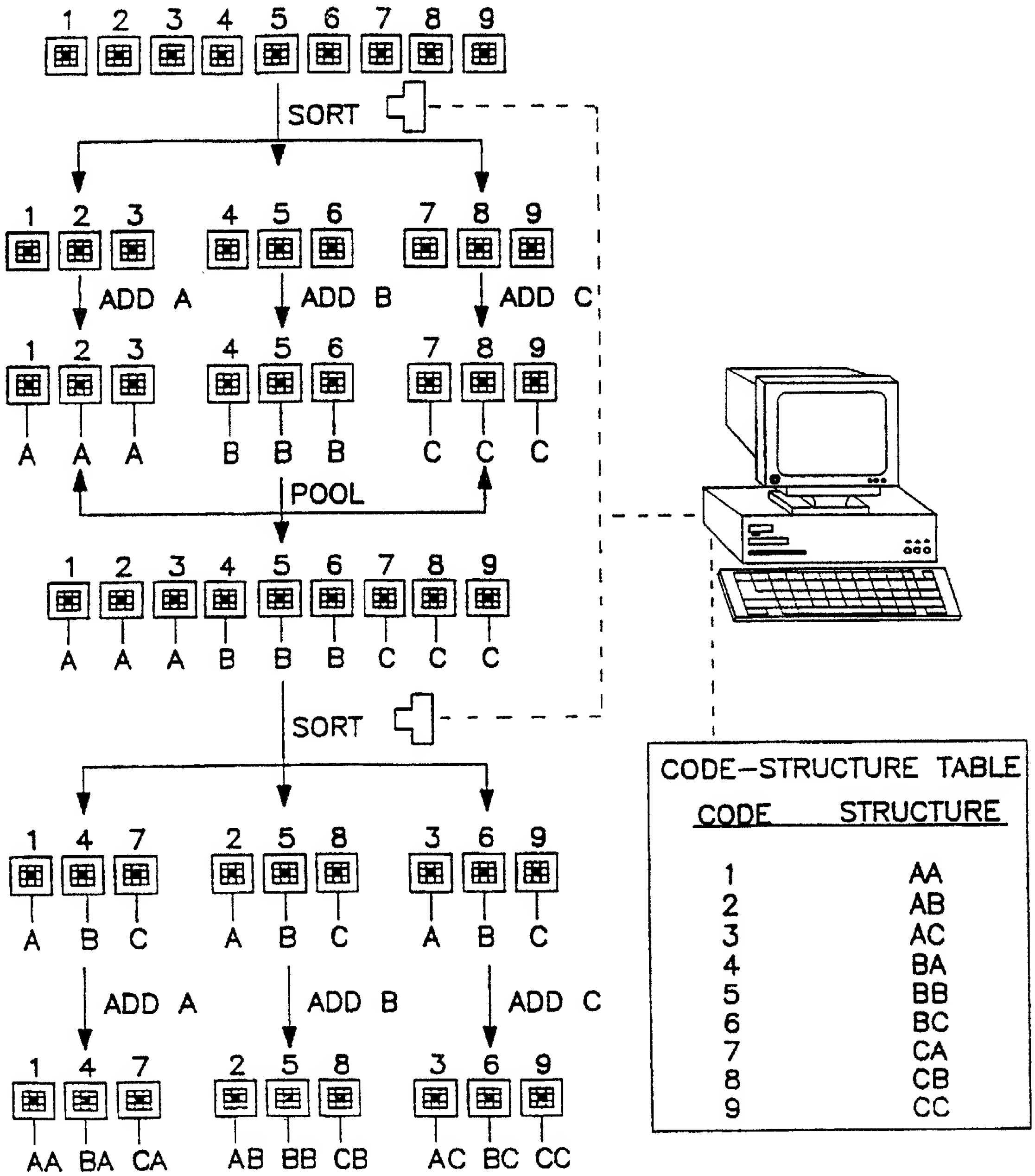


FIG. 33B



CODE–STRUCTURE TABLE

CODE	STRUCTURE
1	AA
2	AB
3	AC
4	BA
5	BB
6	BC
7	CA
8	CB
9	CC

FIG. 33C

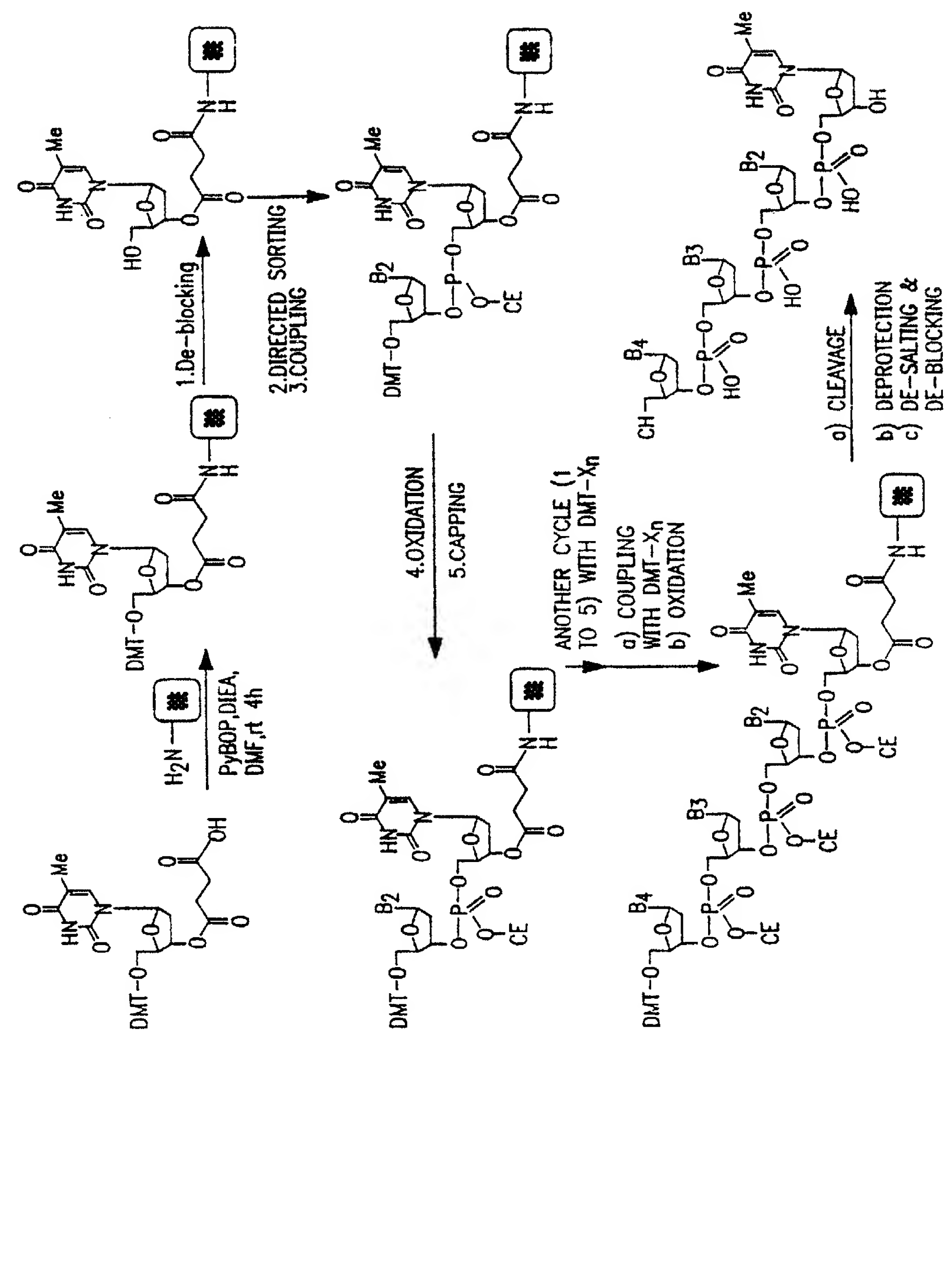

DMT-O
HN
Me
OH
H_2N
PyBOP,DIEA, DMF,rt 4h
1.De-blocking
HO
N
H
2.DIRECTED SORTING
3.COUPLING
B_2
CE
4.OXIDATION
5.CAPPING
ANOTHER CYCLE (1 TO 5) WITH DMT-X_n
a) COUPLING WITH DMT-X_n
b) OXIDATION
B_3
B_4
a) CLEAVAGE
b) DEPROTECTION
c) DE-SALTING & DE-BLOCKING
CH
HO

FIG. 33D

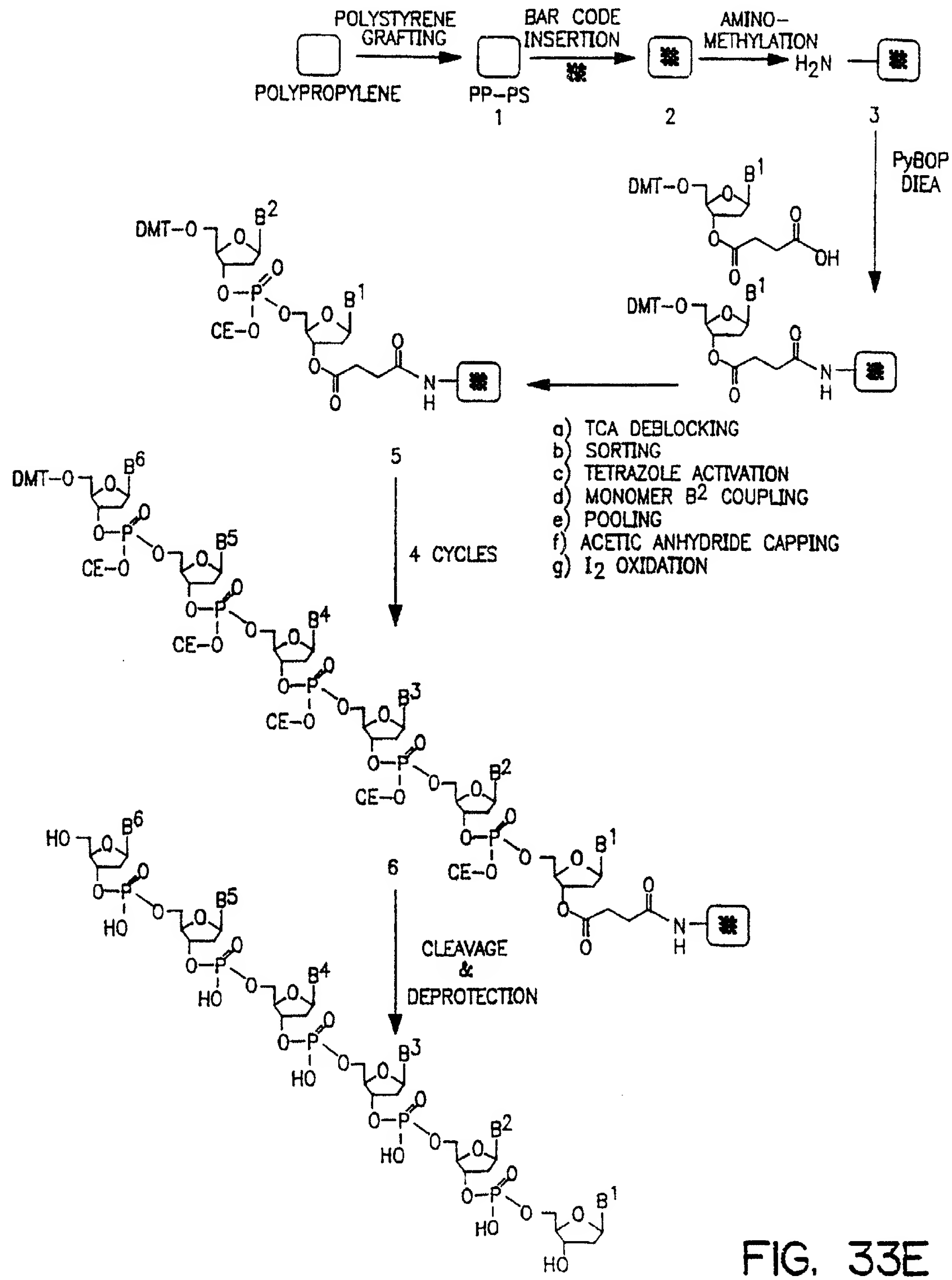

POLYSTYRENE GRAFTING
BAR CODE INSERTION
AMINO-METHYLATION
POLYPROPYLENE
PP-PS
1
2
3
PyBOP DIEA
DMT-O
5
4 CYCLES
a) TCA DEBLOCKING
b) SORTING
c) TETRAZOLE ACTIVATION
d) MONOMER B2 COUPLING
e) POOLING
f) ACETIC ANHYDRIDE CAPPING
g) I2 OXIDATION
6
CLEAVAGE & DEPROTECTION

FIG. 33E

SCHEME 1

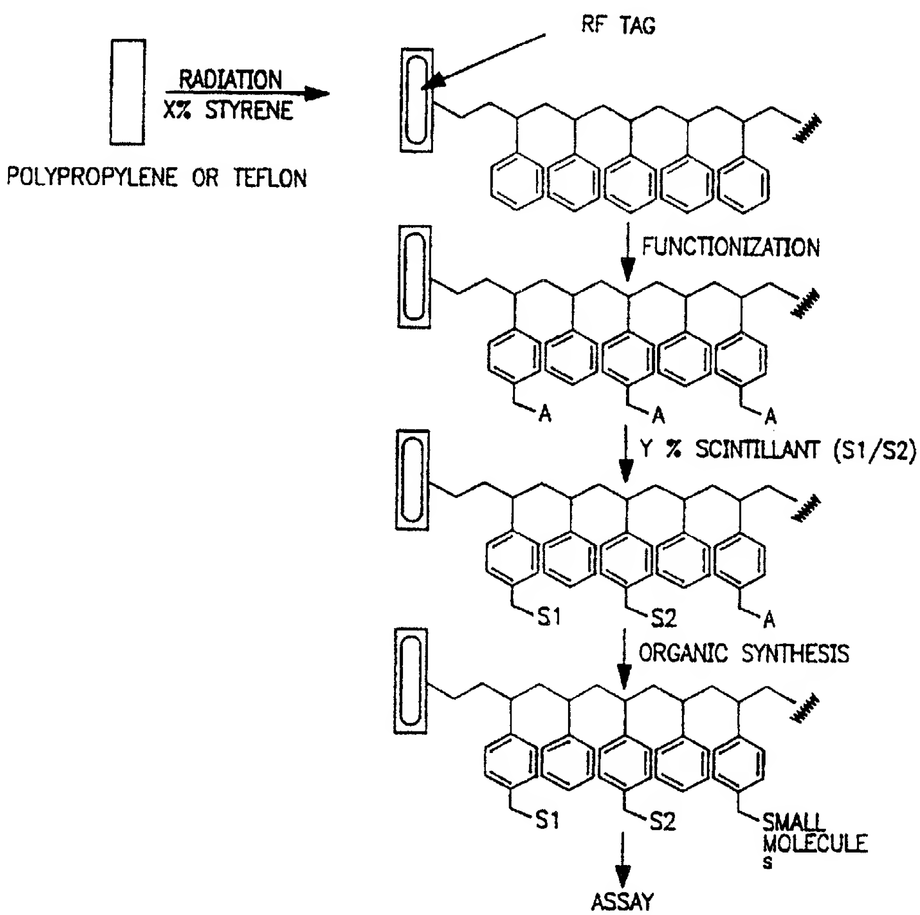


FIG. 34A

SCHEME 2

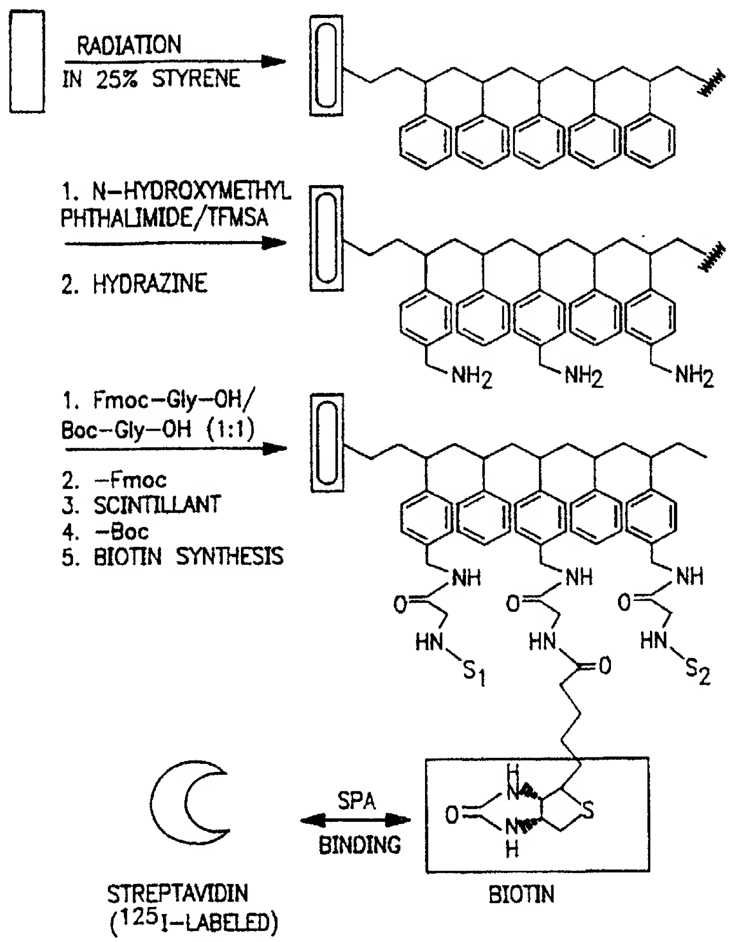


FIG. 34B

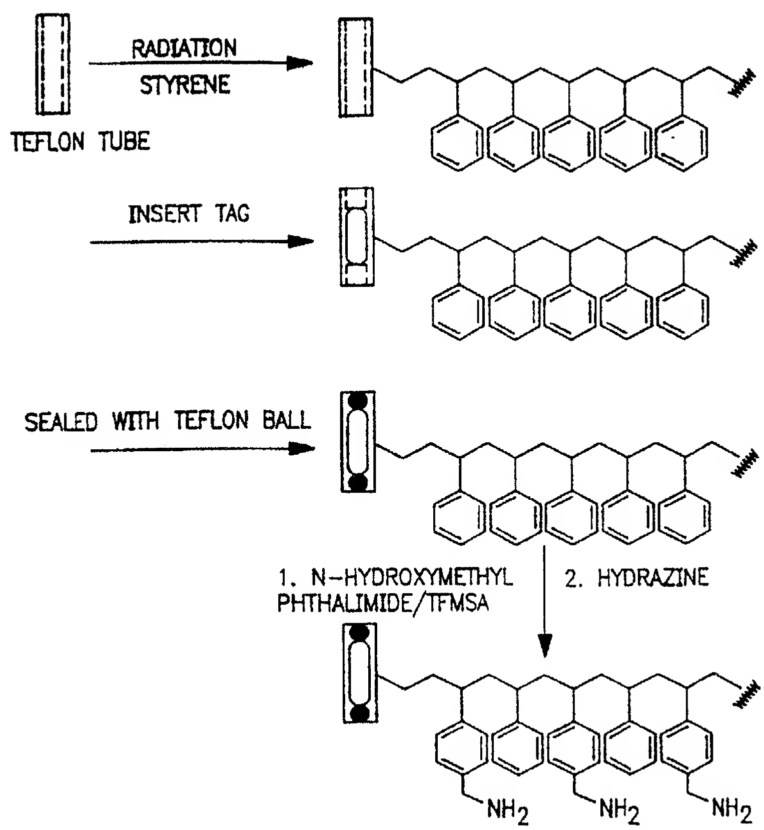

RADIATION
STYRENE
TEFLON TUBE
INSERT TAG
SEALED WITH TEFLON BALL
1. N-HYDROXYMETHYL PHTHALIMIDE/TFMSA
2. HYDRAZINE
NH2
NH2
NH2

FIG. 34C

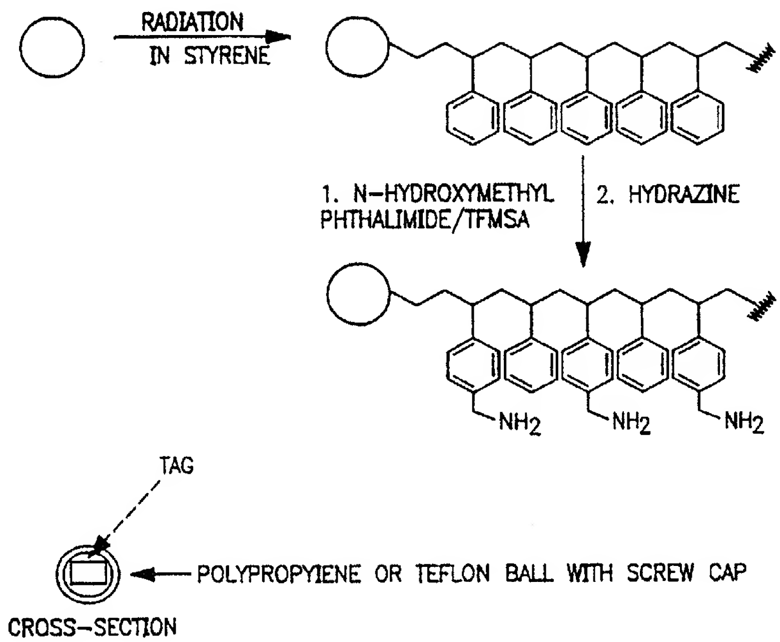
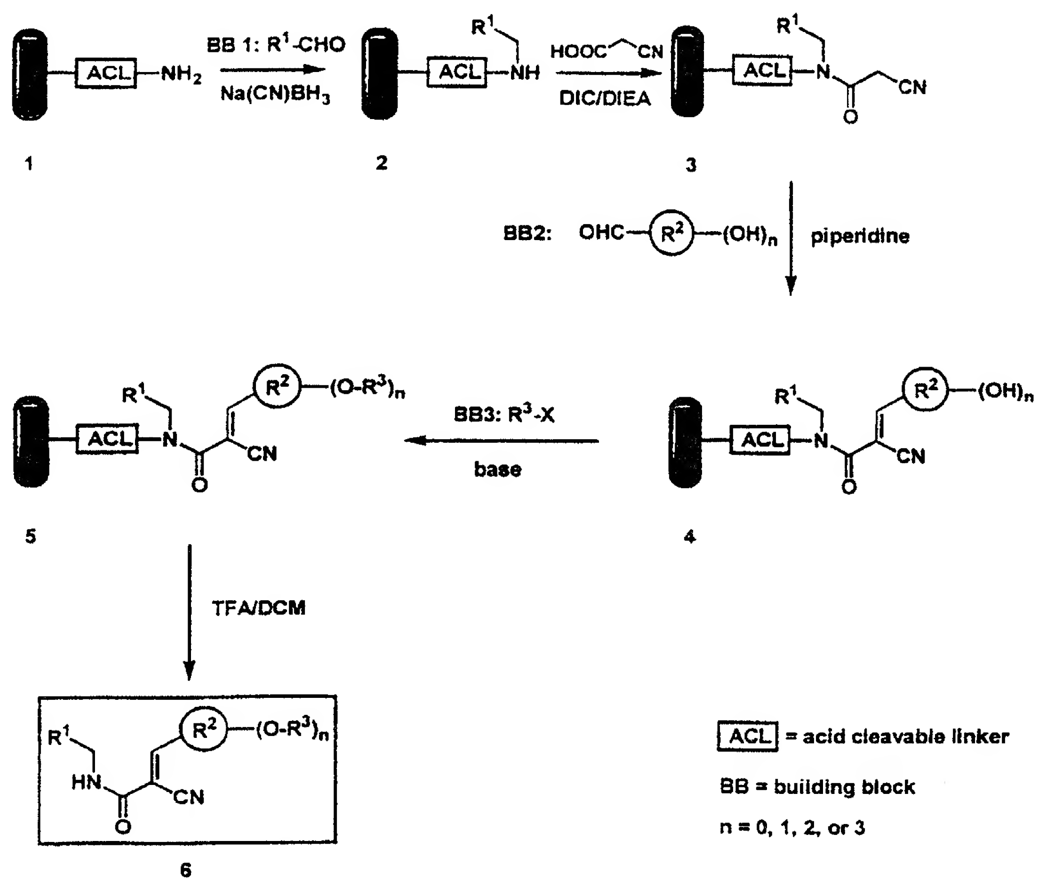

RADIATION
IN STYRENE
1. N-HYDROXYMETHYL PHTHALIMIDE/TFMSA
2. HYDRAZINE
NH2
NH2
NH2
TAG
POLYPROPYIENE OR TEFLON BALL WITH SCREW CAP
CROSS-SECTION

FIG. 34D

FIG. 35

Scheme I



Building block definitions:

BB1:	R^1-CHO	any aldehydes preferably aromatic and heterocyclic aldehydes
BB2:	OHC-R^2-$(OH)_n$ (n = 0, 1, 2, 3)	any aldehydes preferably aromatic and heterocyclic aldehydes more preferably aromatic and heterocyclic aldehydes with one or more hydroxy groups
BB3:	R^3-X (X = leaving group)	any alkylating and acylating reagents preferably alkylating and acylating reagents with aromatic or heterocyclic ring(s) more preferably nothing

Scheme II

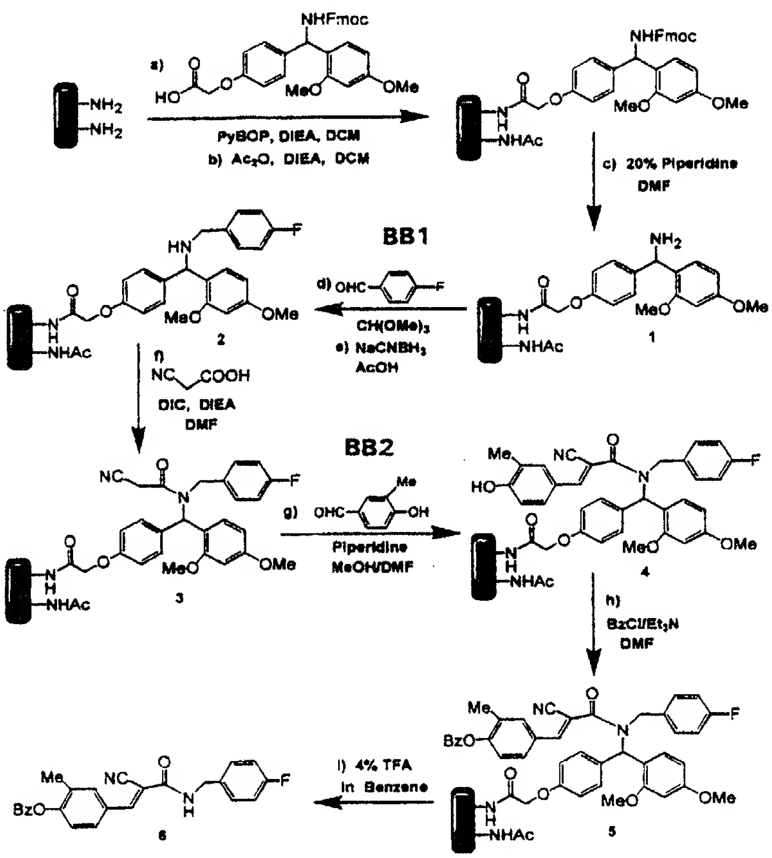


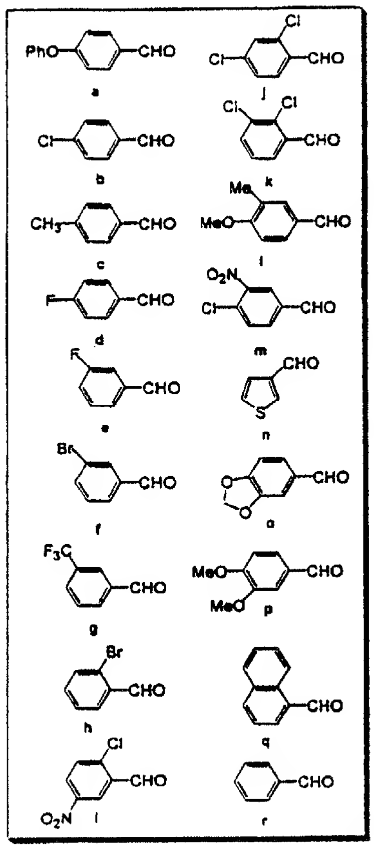
FIG. 36

FIG. 37

EXEMPLARY LIBRARY I CONTAINING 432 MEMBERS

BUILDING BLOCKS

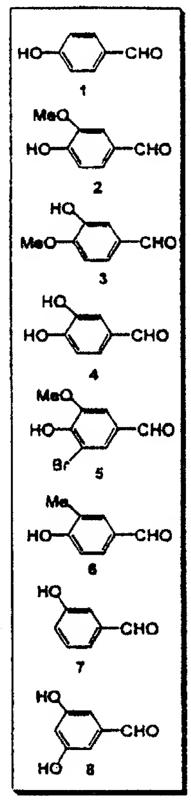
BB1



R^1CHO

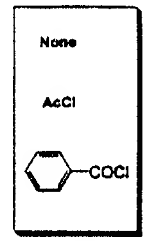
Building blocks for reductive alkylation

BB2



Building blocks for Aldol condensation

BB3



R^3X

Building blocks for estification

Library size: 18 x 8 x 3 =432

Building Block definitions:

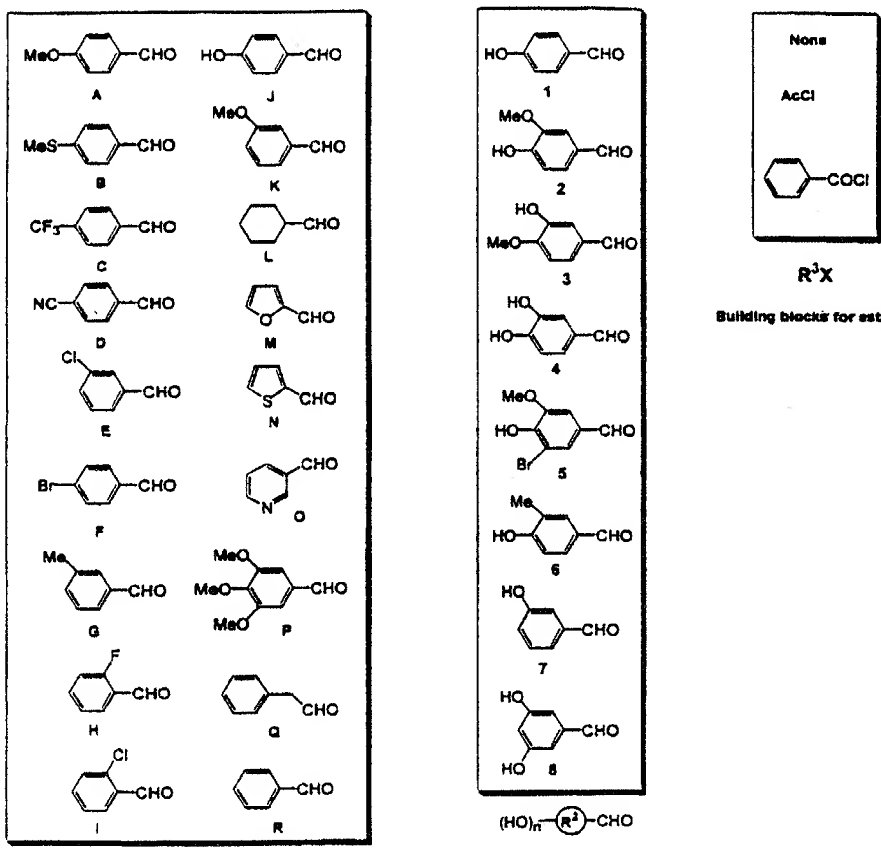
R^1-CHO	any aldehydes, preferably aromatic and heterocyclic aldehydes
OHC-R^2-$(OH)_n$	n = 0,1, 2, or 3, any aldehydes, preferably aromatic and heterocyclic aldehydes, more preferably aromatic and heterocyclic aldehdyes with one or more hydroxy groups
R^3-X	any alkylating and acylating reagents (X = leaving groups), preferably alkylating and acylating reagents with aromatic or heterocyclic ring(s), more preferably no reagents at all.

FIG. 38

EXEMPLARY LIBRARY II CONTAINING 432 MEMBERS

BUILDING BLOCKS

BB1 BB2 BB3



R^1CHO

Building blocks for reductive alkylation

$(HO)_n-(R^2)-CHO$

Building blocks for Aldol condensation

R^3X

Building blocks for estification

Library size: 18 x 8 x 3 = 432

Building Block definitions:

R^1-CHO	any aldehydes, preferably aromatic and heterocyclic aldehydes
OHC-R^2-$(OH)_n$	n = 0,1, 2, or 3, any aldehydes, preferably aromatic and heterocyclic aldehydes, more preferably aromatic and heterocyclic aldehdyes with one or more hydroxy groups
R^3-X	any alkylating and acylating reagents (X = leaving groups), preferably alkylating and acylating reagents with aromatic or heterocyclic ring(s), more preferably no reagents at all.

FIG. 39A
EXEMPLARY LIBRARY III
BUILDING BLOCKS BB1 FOR REDUCTIVE ALKYLATION FOR 10K MEMBER LIBRARY

R^1-CHO

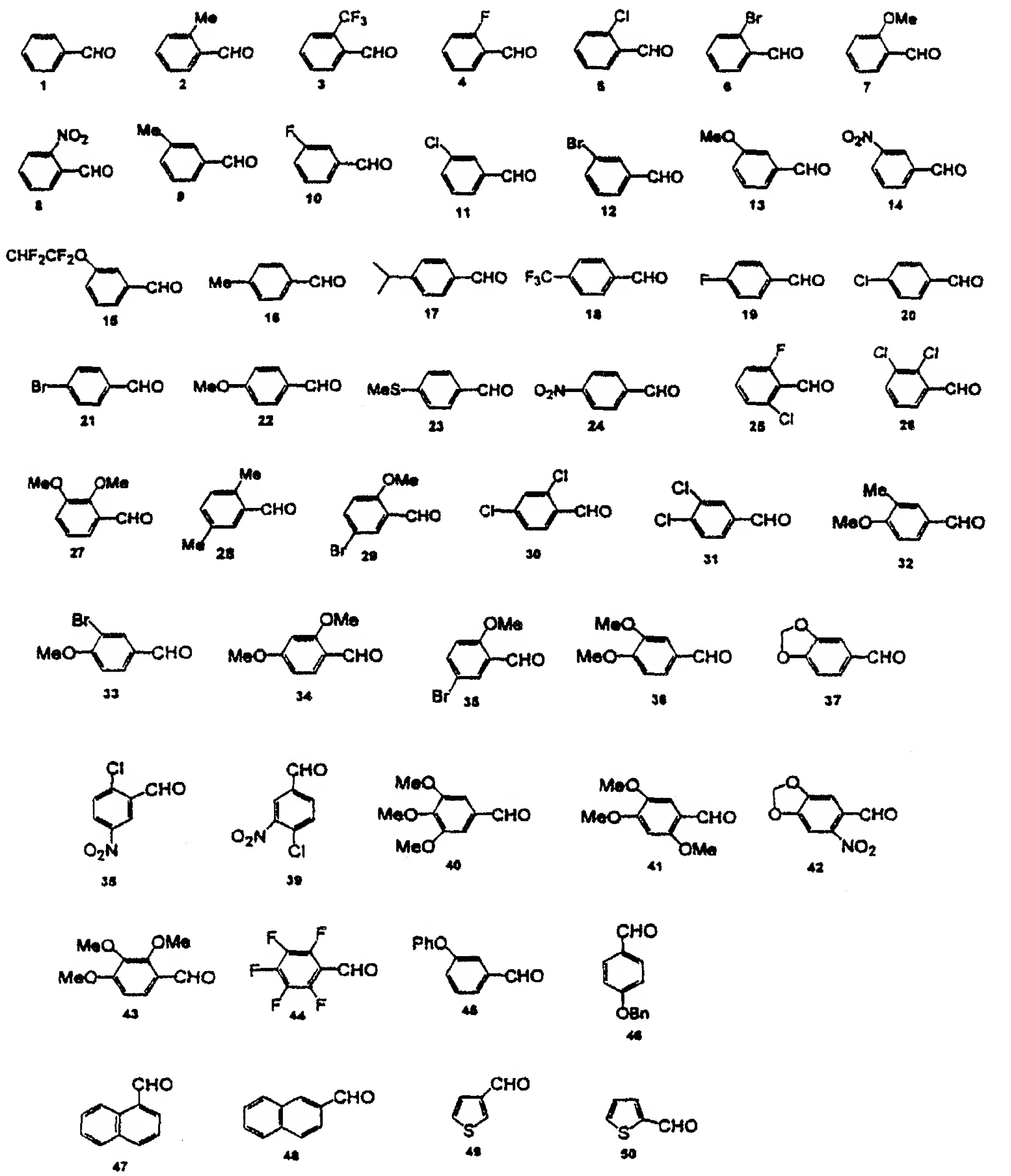


FIG. 39B
EXEMPLARY LIBRARY III
BUILDING BLOCKS BB2 FOR ALDOL CONDENSATION FOR 10K MEMBER LIBRARY

$OHC\text{-}R^2\text{-}(OH)_n$

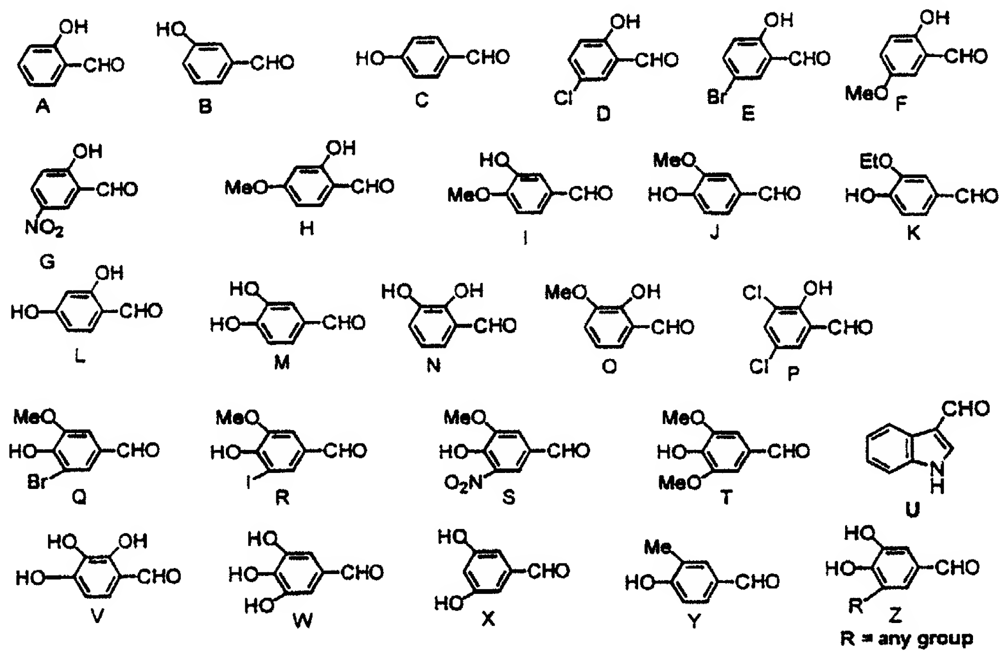
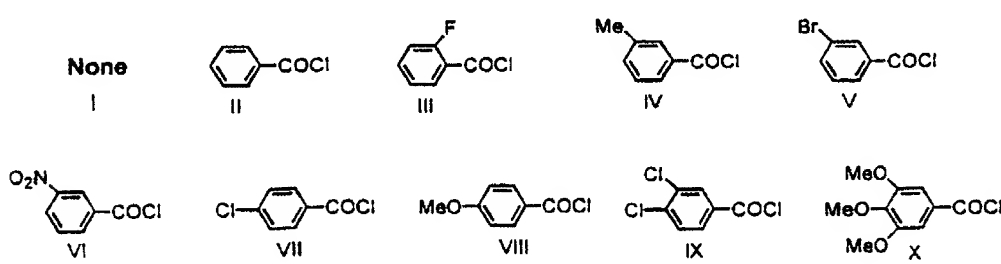


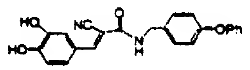
FIG. 39C
EXEMPLARY LIBRARY III
BUILDING BLOCKS BB3 FOR ESTERIFICATION FOR 10K MEMBER LIBRARY

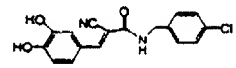
R^3X

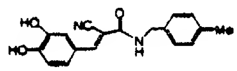


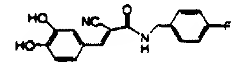
Building block (R^1-CHO, $OHC\text{-}R^2\text{-}(OH)_n$, and R^3X) definitions are same as these on pages 2, 4 and 5.

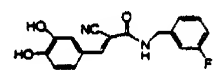
FIG. 40
EXEMPLARY COMPOUNDS

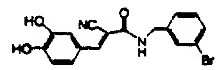
a4 

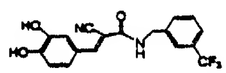
b4 

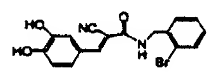
c4 

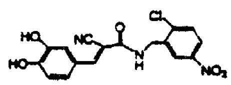
d4 

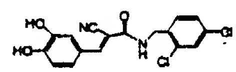
e4 

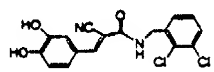
f4 

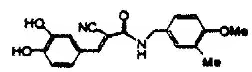
g4 

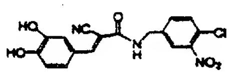
h4 

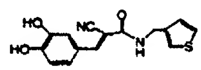
i4 

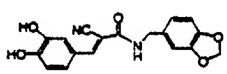
j4 

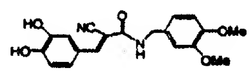
k4 

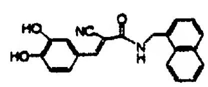
l4 

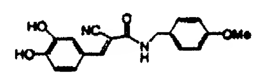
m4 

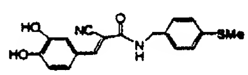
n4 

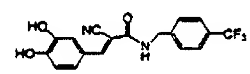
o4 

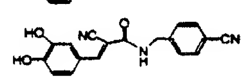
p4 

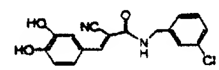
q4 

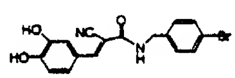
A4 

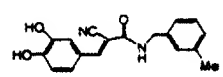
B4 

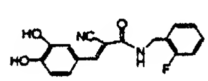
C4 

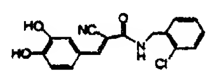
D4 

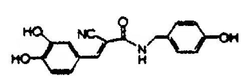
E4 

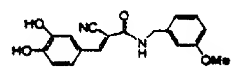
F4 

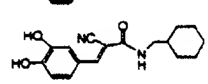
G4 

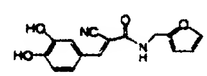
H4 

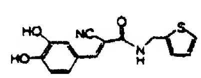
I4 

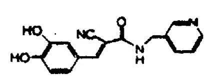
J4 

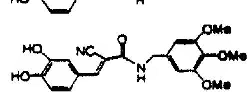
K4 

L4 

M4 

N4 

O4 

P4 

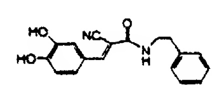
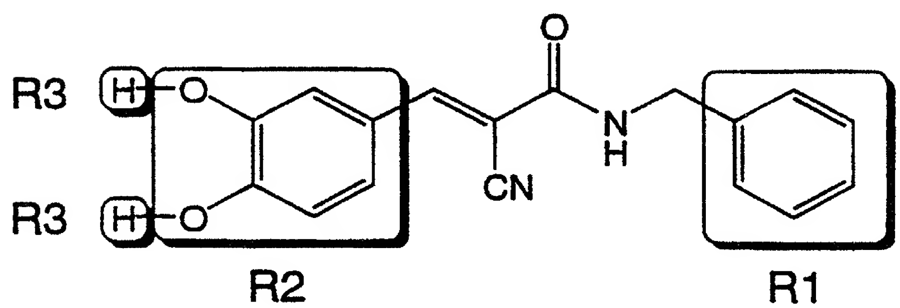
Q4 

FIG. 41
CONCLUSIONS FROM SAR FROM AG490 LIBRARY

based on tyrosine kinase inhibition of p60$^{c\text{-}src}$ with 40 tyrphostins

R^2 preferably contains a 3,4-dihyroxyphenyl group

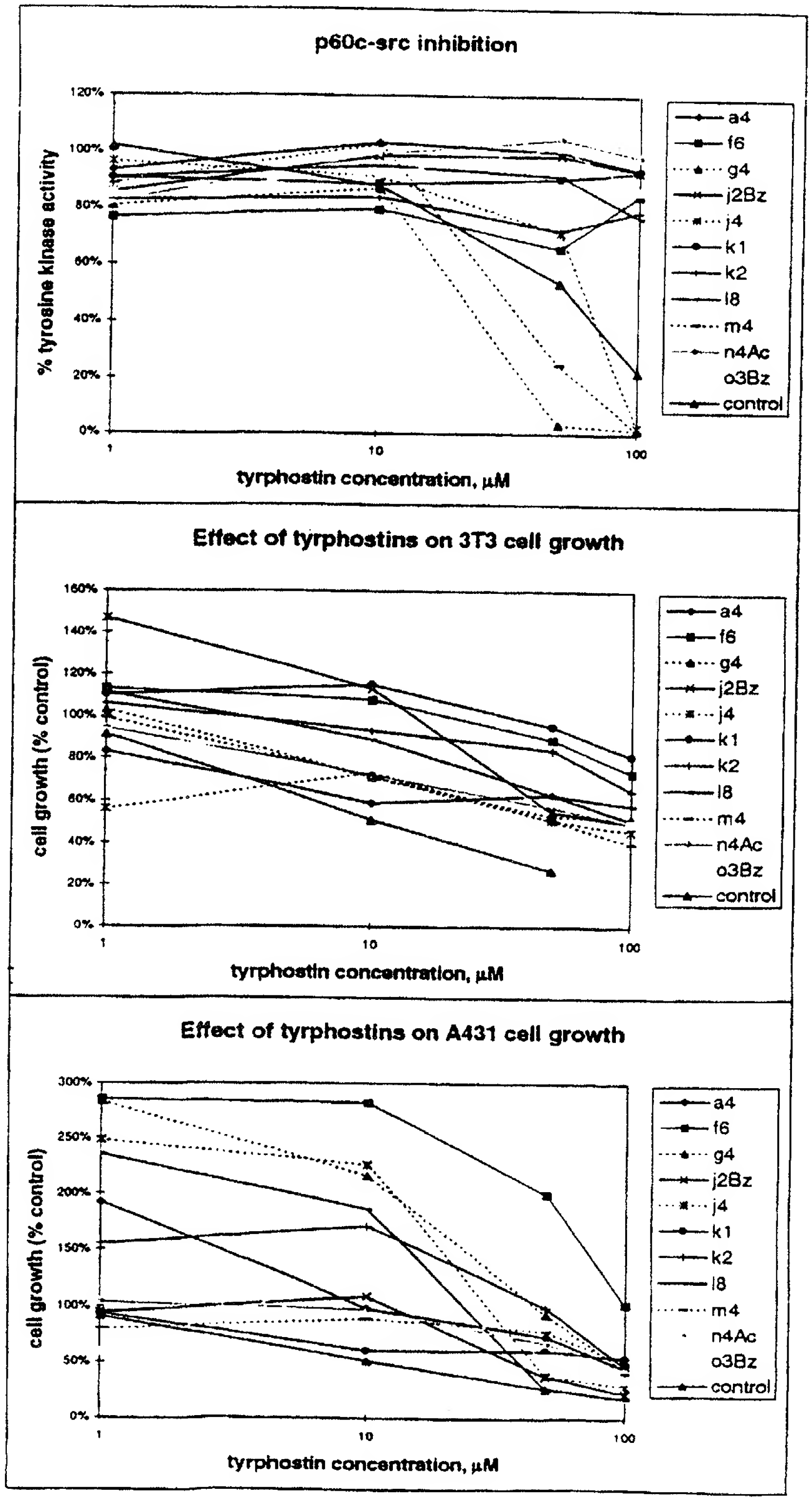


R^3 preferably does not accommodate an ester substitution

R^1 can accommodate a variety of substituents, but is preferably substituted at the meta position

FIG. 42

selected compounds from 40 tyrphostins screened



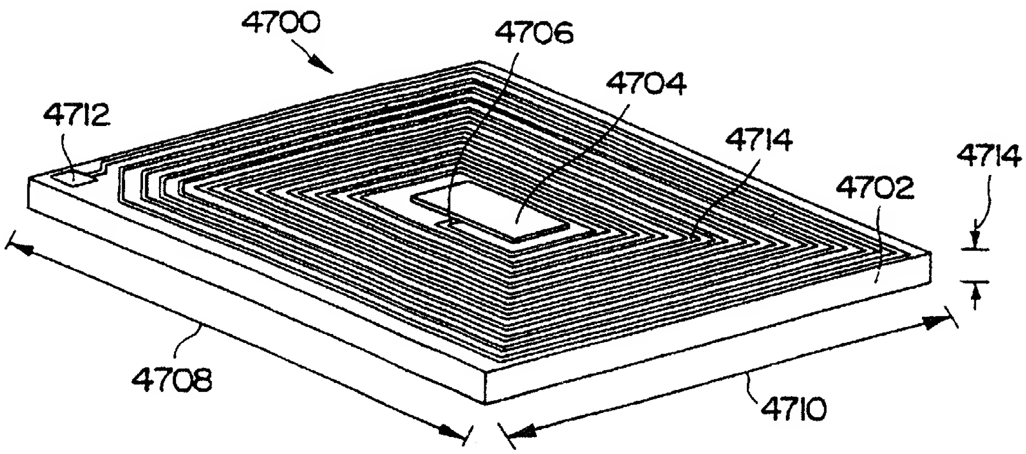

4700
4706
4704
4712
4714
4714
4702
4708
4710

FIG. 43

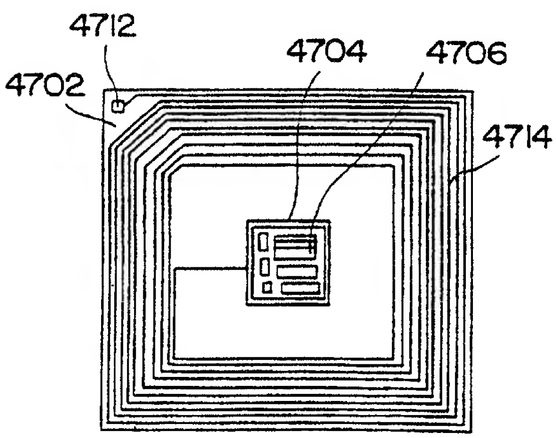

4712
4704
4706
4702
4714

FIG. 44